UGC – MINOR RESEARCH PROJECT

Final Report and Utilization Audited Certificate

Ref. No : F. MRP 4832/14 (UGC - SERO) Dated : March 2014

Submitted to

ज्ञान-विज्ञान विमुक्तये

University Grant Commission

South Eastern Regional Office (SERO)

HYDERABAD

Project title : " *Effect of synthetic pyrethroids Cyfluthrin and Fenvalerate on the Biochemical constituents of Gambusia affinis (mosquito fish)* "

OSMANIA COLLEGE KURNOOL LEAD KINDLY LIGHT

Submitted by

Dr.S.Mohammed Ghouse , M.Sc, Ph.D, SLET.
Reader
Department of Zoology
Principal Investigator
Osmania College(A) , Kurnool – 518001 (A.P)

DECLARATION AND CERTIFICATE

I hereby declare and certify that, the Minor Research Project entitled "**Effect of synthetic pyrethroids Cyfluthrin and Fenvalerate on the Biochemical constituents of *Gambusia affinis*** " (**mosquito fish**) vide UGC F.No MRP - 4832/14 (SERO/UGC) dated March 2014 is a bonafide record of research work carried out by me during the year 2014- 2016. Further certify that the work presented in the report is original and carried out according to the plan in the proposal and guidelines of the University Grants Commission.

Date : **Principal Investigator**

(Dr S.MOHAMMED GHOUSE)

CONTENTS

Particulars	Page No.
Acknowledgements	i
List of Tables	ii
List of Figures	iii
List of Abbreviations and Symbols	iv
Chapter 1- Introduction	1-12
Chapter 2- Materials and Methods	13-33
Chapter 3- Results and Discussion	34-90
Chapter 4- Summary and Conclusion	91-94
References	95-127
List of Publications	128 - 147

ACKNOWLEDGEMENTS

I express my sincere thanks to ***Madam Azra Javeed Sahiba****, Secretary and Correspondent, Osmania Group of Institutions, Kurnool and* ***Dr. M Silar Mohammed*** *and* ***Dr. Syed Makbul Hussian****, Principals, Osmania College, Kurnool ,IQAC Coordinator* ***S.Samiuddin Muzammil*** *for their co-operation and encouragement .*

I would like to express my deep appreciation to my parents and my wife Samiunnisa & children Shoiab Tanver Md Rafi and Sana Gousia Nadia Parveen , Chotu (Little Singham) and friends for their constant cooperation during entire period of work.

I express my deepest sense of gratitude and indebtedness to Prof.Indira Head of the Department of Zoology, Sri Krishna devaraya University for her support during the course of the work. I express my sincere thanks to all the staff members of Department of library Acharya Nagrjuna University , Guntur, Sri Venkateswara University, Tirupati for their help, kind co-operation for completion of this work.

I would like to give thank to Expert committee members of the my college and all the staff members of Department of Zoology, Osmnaia College ,Kurnool for their help'

The financial assistance provided by the ***UGC Southern Eastern Regional Office, Hyderabad*** *in the form of Minor research Project is gratefully acknowledged.*

Last but not least I wish to offer my heartfelt thanks to all, whose names could not be included, but will be fondly remembered.

Date :

Kurnool **Dr S.MOHAMMED GHOUSE**

LIST OF TABLES

Table No.	TITLE	Page No.
1	Median lethal concentration LC_{50} values for the fish *Gambusia affinis* exposed to different concentrations of cyfluthrin and fenvalerate for different at different periods.	23
2	Variations of RBC (X $10^6/mm^3$), WBC (X $10^3/mm^3$, Haemoglobin (Hb) g/dL., and PCV (%) ,Blood glucose (mg/dL),values values in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	37
3	Variations of Proteins (mg /g wet wt. of tissue) content in the in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	42
4	Variations of amino acid (µg/mg wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	47
5	Variations of glucose (mg/g wet. wt. of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	51
6	Variations of values Succinate dehydrogenase (SDH) (µg formazon/mg protein/hr) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	56
7	Variations of values Lactate dehydrogenase (LDH) (µg formazon/mg protein/hour) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	60
8	Variations of glucose-6 phosphatase (µg Pi liberated/mg protein/h.) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	63
9	Variations of Acetylcholinesterase (AChE) (umole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	67
10	Variations of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	72
11	Variations of Ribonucleic acid (RNA) (mg/g wet. wt of tissue) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	76

LIST OF FIGURES

S. No.	TITLE	Page No.
1	Fig 1. Median lethal concentration LC_{50} values for the fish *Gambusia affinis* exposed to different concentrations of cyfluthrin and fenvalerate for different at different periods	24
2	Fig. 1 Changes in the level of blood RBC (X $10^6/mm^3$), WBC (X $10^3/mm^3$, Haemoglobin (Hb) g/dL., and PCV (%) , Blood glucose (mg/dL),values values in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	38-39
3	Fig. 2 : Changes in the level of Proteins (mg /g wet wt. of tissue) content in the in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	43-44
4	Fig. 3 : Changes in the level of amino acid (µg/mg wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	48-49
5	Fig. 4 : Changes in the level of glucose (mg/g wet. wt. of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	52-53
6	Fig. 5 : Changes in the level of Succinate dehydrogenase (SDH) (µg formazon/mg protein/hr) activity in *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	57
7	Fig. 6 : Changes in the level of Lactate dehydrogenase (LDH) (µg formazon/mg protein/hr) activity in *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	61
8	Fig. 7 : Changes in the level of glucose-6 phosphatase (µg Pi liberated/mg protein/h.) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	64
9	Fig. 8 : Changes in the level of Acetylcholinesterase (AChE) (u mole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	68-69
10	Fig. 9 : Changes in the level of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	73
11	Fig. 10 : Changes in the level of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours	77

A B B R E V I A T I O N S

AChE - **Acetylcholinesterase**

APHA - ***American Public Health Association***

ANOVA - ***Analysis of Variance***

EPA - ***Environmental Protection Agency***

FAA - ***Free Amino Acid***

Hb - ***Haemoglobin***

LC_0 - ***Maximum Tolerable Concentration***

LC_{50} - ***Median Lethal Concentration***

LC_{100} - ***Absolute Lethal Concentration***

LDH - ***Lactate dehydrogenase***

mg/L - ***Milligram per liter***

µg/L - ***Microgram per liter***

PCV - ***Packed Cell Volume***

Ppm - ***Parts per million***

RBC - ***Red Blood Corpuscles***

SD - ***Standard Deviation***

SDH - ***Succinate dehydrogenase***

WBC - ***White Blood Corpuscles***

WHO - ***World Health Organization***

CHAPTER - 1

INTRODUCTION

GENERAL INTRODUCTION

India is primarily an agro-based country with more than 60–70% of its population dependent on agriculture. Rapid industrialization and successful green, white and blue revolutions have necessitated a large variety of chemicals usage resulting environmental pollution which has become not only a national but a global problem. More than 30% of its agricultural productivity is lost due to the to pests . Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. Under the U.S. Federal Environmental Pesticide Control Act, the term Pesticide has been defined to include any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. (Gupta and Satankhe, 1985).

Pesticides have economic and public health benefits and have been used over the years for the control of vector borne diseases such as malaria, rocky mountain fever, and domestic pest (Jacob S.S., 1982). These pesticides by different routes finally reach the aquatic systems affecting the aquatic organisms (santhakumar ,.1999). Pollution is now the important limiting factor for man. The growing population together with the rapid industrialization has made the problem more serious. Excess use of insecticides to get more agriculture yield and spraying of pesticides improperly in agricultural fields cause these chemicals to accumulate in the field, along with rain water these pesticides finally reach the natural water systems like ponds, streams, lakes and rivers., it will ultimately lead to alteration in the ecosystem (Odum, 1971).

In the early 18th century the Caucasian tribes have used Pyrethrum flowers to control body lice. The insecticidal properties of Pyrethrum flowers (*Genus Chrystanthemum)* most commonly *C. cinnerariae folium* flowers have been recognized since the middle of the last century. In the 19th century it was

introduced in Europe (1982), the United States (1876) Japan, Africa and South America. Population and pollution are the most important problems faced by the globe . The population explosion and urbanization together with rapid industrialization have led to the contamination of air, water and land with chemicals .

The population goes on increasing year by year, in order to feed this population a tremendous pressure is put on the land. Countries are using more pescticides to enhance the agricultural productivity. Pesticide chemicals are being used increasingly for the protection of crops against weeds, pests and diseases and have become part and parcel of agrochemical technology. Around 1940 the era of synthetic organic pesticide chemicals began. These chemicals were so successful in controlling pests that progressed so rapidly as to start a new era of pest control that led to a burst of pesticide inventions. Over the past forty years pesticides have become an integral and indispensable part of world agriculture.

The pesticides usage is desirable for the control of pests on the one hand and on the other hand, these are causing environmental pollution (Zitko., 1975; Pandey., 2000). Man in the process of development polluted his surroundings environment such as rivers, seas, other aquatic bodies, land, air by the increased use of pollutants . Pesticides are introduced to combat the vector borne diseases of the crops to increase the productivity. Pesticides have both desirable and undesirable effects.

Pesticides are hazardous to the biodiversity including the man .Pesticides have detrimental effects on behaviour ,reproduction, growth, hormonal regulation etc of the organisms .These pesticides through the process of biomagnification increase their concentration at the top trophic level of the food chain, including the in humans.

It is necessary to understand how the pesticides are entering into the water bodies .there are several ways i.e

1) Direct application of pesticides as spray or granules to control water inhabiting pests(Hint and Bischoff , 1960)
2) Heavy rain fall immediately after the application of pesticides
3) Agricultural wastes, accidents , and spills etc
4) Surface run off and sediment transport from treated soil (Peterle and Gillet 1970, Bauman, 1981)
5) Spray drift for normal agriculture operations(Westlate and Gunther,1966)
6) Waste disposal by the pesticides processing units (Chowdary et.al)

Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. Based on their toxicological properties in vertebrates and invertebrates . The primary mode of action of pyrethroids in vertebrates and invertebrates is the disruption of voltage sensitive sodium channels in the neurons. By delaying the closing of the ion channel, multiple action potentials result, leading to multiple firing of the neurons, and neurological disruption. Unlike the earlier organophosphate or carbamate insecticide chemistries, pyrethroids do not inhibit acetyl cholinesterase. The toxic effects of pyrethroids are mediated by preventing the closure of the voltage-gated sodium channels in the axonal membranes. Pesticides are called axonic excitotoxins.

Pyrethroids having high efficiency with low toxicity and easy biodegradability are preferred over other insecticides. (Kale, M.N., S. John Rathore and D. Bhatnagar ,. 1999).). Initially pyrethroids were used for the control of household, agricultural insect pests as well as in industrial, stored products and veterinary applications, for example in houses, restaurants, and aircrafts food and for pre-harvest treatment of expensive food crops and in public health (Stevenson, 1963).The synthetic pyrethroids act as excitatory nerve poisons, thought to poison by interfering with the ionic permeability of nerve cell membranes. Through a better understanding of toxicology and toxicity testing in relation to these

biochemical parameters in non-target organisms like fish we may be able to contribute to such an inquiry and hence the present work**.**

Various pesticides that are commonly used in pest control programmes are generally grouped under 4 heads: They are:

(1) Chlorinated hydrocarbon, the 1st generation insecticides [aldrin, DDT, chlordane, dicofol (kelthane), dieldrin, endrin, endosulphon, heptachlor, kepone, lindane, methoxychlor, mirex, and toxaphene];

(2) The second generation organophosphate insecticides include, parathion, malathion, diazinon, monocrotophos etc.,

(3) The third generation insecticides are exemplified by carbamates like, carbaryl, carbofuran, propuxur etc., and

(4) The fourth generation insecticides are the pyrethroids

I. a**) Organochlorine (hydrocarbon) insecticides**

The best example is DDT i.e dichlorodiphenyltrichloroethane invented during 1939, Muller in 1948 (Muller, 1946) DDT the modern insecticide widely used in 1950 along with the other discovered insecticides to eradicate the population of pests. the organochlorine insecticides have become concern to the ecologists as they last long in the nature, and their biomagnifications in the bodies of animals and plants. Organochlorine insecticides in the aquatic medium contribute both direct and indirect hazards to fishes as tissues of the body of fishes are lipophilic in nature. and also to those organisms that feed on fishes

II. **Development of organophosphorous (O.P.)** - the second generation compounds : Due to the toxicity and persistence, bioaccumulation in the environment the organochlorine insecticides are replaced by rganophosphorus which are less persiastant ,less lipophilic (DDT) .The organophosphorus compounds are toxic in that they irreversibly inhibit certain enzymes of the neuromuscular system. Because of their cholinesterase

inhibition and public health problems arising due to spillage, further research led to the coming of the III generation pesticides, the carbamates.

Ill. Carbamates

Carbamates are derivatives of carbamic acid .The third generation insecticides are the carbamates which are commonly used for pest control instead of organophosphates. They kill the organisms by inhibition of acetylcholinesterase enzyme activity. The carbamates are not broad- spectrum insecticides, and some of the common household insect pests such as the housefly and German cockroach are relatively immune (O'Brien, 1967).

IV. Pyrethroids - the fourth generation pesticides

The further search for the non persistence ,less bioaccumulation and less toxicity compared to above three types of pesticides leads the development of Pyrethroids which are natural i.e pyrethrum insecticidal factor in local pyrethrum species and synthetic Pyrethroids i.e Allethrin .

There are two types of pyrethroids. Type I are those pyrethroids which do not have an alpha cyano group and cause repetitive discharges, increase depolarization after potential and slow sodium current during the polarization in the nerve cells. Type II pyrethroids possess alpha cyano group, which causes membrane depolarization without repetitive discharges, do not increase depolarization after potential (Narahashi, 1983).

Fish occupy a prominent position in the field of aquatic toxicology and have been employed in studies concerning ecological health. Fishes are usually exposed to highly contaminated water, which are in areas where wastewater dilution rate is low (Bernet ., 1999). Fish play a vital role in the toxicity testing for ecological effects as the most employed organisms for bio monitoring . In fish, the principle mode of uptake of pesticides is through the gills (Holden, 1965), body surface and gastro-intestinal system (Murphy, 1980), through integument (Bowes, 1972) and by the ingestion of

contaminated food (Macek ,. 1969). However the portals of entry of the pesticides differ from organism to organism. Pesticides contamination in water directly of indirectly results in death of the fish, reduced fish productivity or bioaccumulation of harmful chemicals in edible fish tissue, which finally affect adversely the human health who consume this fish . WHO (1986) described that the toxicant have their way in to the water body as a result of runoff of the residue which is said to be highly hazardous and harmful to fish.

Pesticides accumulate in the tissues of aquatic organisms and plants by biomagnification through adsorption and ingestion .The size, growth rate , surface area, lipid content and age of the organism plays an important role in bioaccumulation. The process of bioaccumulation or biomagnification depends on certain environmental factors like availability of food, oxygen levels and temperature etc,.

Pesticides use in India

Pesticides are used around 1000 B.C. E. Homer referred to the use of sulfur to fumigate homes and by 900 C.E the Chineese were using arsenic to control garden pests. Many centuries ago sophisticated agricultural societies used chemicals for pest control (Younos and Weigmann, 1988). Sulphur was used for disease and insect control before 1000 BC and the use of arsenic was advocated around 79 A.D (Cremlyn, 1978). After China, India is the second largest producer of pesticides in Asia and with 12th ranks globally. India started the production of BHC in 1952 with the establishment of a plant near Calcutta. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat. In India the usage pattern of the of pesticide is different from the world in general.

Globally 44% of the pesticide is used as inscecticide , where as in india 76% of the pesticide used as insecticide . (Mathur , S.C. 1999). In India as much as 70% of the chemical formulations employed in agricultural

practices are believed to affect non-target organisms and to find their way to fresh water fish, ultimately polluting them (Bhatnagar MC, , Bana AK., 1992).

Reports of pesticide residues / poisoning in India

In India the residues of pesticides in food, raw agricultural commodities and components of the environment have not so far been studied extensively. Still a few reports show the presence of pesticide residues in essential commodities. Research in Tarai area of Uttar Pradesh shows an average DDT content of 0.46 + 0.35 ppm in vegetables (Visweswaraiah.,, 1975). BHC levels in some vegetables in Mysore (India) varied from 10.5 to 20 ppm (Visweswaraiah and Jayaram, 1972). BHC levels in Hasan vary from 3.68 to 15.5 ppm (Manjumder and Krishnamurthy, 1963). Research in Tarai area showed an average DDT content of 0.845 + 0.51 ppm in dairy concentrates; 0.39 + 0.028 ppm in poultry eggs, 0.58 + 0.64 ppm in hays and other feeds and 0.0032 + 0.002 ppm in milk . (Viswesaraiah,. 1975). (Joia, 1979) reported contamination of BHC and HCH ranging from 1 to 5 ppm in wheat flour in Punjab. The incident of pesticide poisoning in agricultural workers of Handigodu came to light in December, 1977; when they were reported to have suffered from painful swelling and immobilization of hip and knee joints ultimately leading to disuse atrophy of lower limbs, due to the long term consumption of pesticide - exposed crabs and fish (NIN, 1977).

The death of 150 persons in Kerala due to the consumption of malathion mixed wheat has also been reported (The Hindu, 1985).. David (1985) has cited two epidemics of epilepsy in Sitapur of Uttar Pradesh due to BHC poisoning. A rapid survey conducted by the Central Fisheries Extension Centre, Hyderabad revealed the mortality of large variety of fish during 1973 due to pesticides (Hingorani ., 1973). According to a report of Central Bureau of Investigation (CBI), Government of India, as many as 4,536 persons died in 1965 alone on account of carelessness in handling poisonous substances (Visweswaraiah , 1975).

Insecticides which are used commonly in agriculture cause the death of the target insects within a few hours by inflicting irrevocable damage on the peripheral and central nervous systems (Leake . 1985; Reddy and Philip 1994). The increasing use of pesticides in agriculture including commercial and household production of vegetables for the control of pests causes chemical pollution of aquatic environment. Pyrethroids are generally safe in mammals and birds but are highly toxic to fish and aquatic invertebrates The chemical pollution causes potential health hazards to live stock, especially to fish, frogs, birds and mammals.

The pyrethroids are widely used in field pest control and household use, and as veterinary and human pediculicides and are among the most potent insecticides known (Smith and Stratton 1986). Pyrethroids having high efficiency with low toxicity and easy biodegradability are preferred over other insecticides. (Kale, M.N., S. John Rathore and D. Bhatnagar ,.1999).). The synthetic pyrethroids interfere with ionic permeability of nerve cell membranes act as excitatory nerve poisons . Pesticides are harmful chemicals pose threst to the environment.The contamination of surface waters by pesticides used in agriculture is a problem of worldwide importance (Hill, I. R, (1985). Pesticides contributed considerably to human welfare, their adverse effects on non-target organisms are significant. Sublethal adverse effects may result from exposure of aquatic organisms to insecticides at environmentally relevant concentrations (Das, B. K. and Mukherjee, S. C, 2003), The range of pesticide toxicity is large in aquatic organisms, among different fish species it varies, and the variation depends on the age, sex, size, physiological and biological conditions of individual fish and also the environmental factors (Sprague, J. B, 1969).

Biochemical parameters serve as specific indicators of pesticide for monitoring the pathological status of the treated fish.The potential toxicity of the pesticide will lead to tissue damage and change in behavior . (Latner, A.L., 1975 , Venkataramana G.V ., *2006)*. The detoxification mechanism

present in the body of the organism will work to get rid of foreign substances. If the concentration of toxic substance is higher it results in damage at cellular or even at molecular level, which leads to behavioral, physiological, pathological and biochemical disorders that may prove fatal conditions to aquatic life (Anita Susan T., *1999*, Rathod D.S., *2009,* Satyavardhan K., 2010 , Yaji *A.G., 2011)* .

The number of changes in the biochemistry of the fish was reported as a result of an exposure to pesticides .In order to assess the toxicity of the pesticide biochemical studies are good parameters to observe the metabolic changes in fish (Ghosh T.K., 1986., , Kajare, A., 2000, Tilak , K.S., 2001, and Tilak, K.S., K. Veeraiah , 2001) . Hence, the present investigation was taken up to find out the induced effects of cyfluthrin and Fenvalarate insecticides on biochemical constituents from total body muscles (such as Gill, Liver, Kidney, Brain and muscle) of experimental fish *Gambusia affinis.*

Blood is a fluid connective circulating in the body and it provides one of the methods of communication between the cells of different parts of the body. Blood plays a vital role in the physiological, nutrition and pathological status of an organism. Haematological parameters such as haematocrit, haemoglobin, erythrocytes and white blood cells , PCV etc., can be used as indicators of toxicity and have a broad potential application in environmental and occupational monitoring (Sancho E., 2000; Barcellos L.J.G., 2003). The haemotological screening provides opportunity to investigate the existence of different metabolites and other constituents in the body of animals (Aderemi, F.A., 2004; Doyle, 2006).

Blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions (Olafedehan C.O., 2010). The response of animals to various physiological situations can be assessed by observing the alterations in the blood constituents in accordance to the physiological conditions (Khan T.A., and Zafar, F., 2005). Examining blood for their constituents can provide important information for the diagnosis and

prognosis of diseases in animals (Olafedehan C.O ., 2010). Stresses due to environmental, nutritional and/or pathological factors can later changes in haematological parameters are often used to determine various status of the body. (Afolabi K.D., 2010). They are those parameters that are related to the blood and blood forming organs (Waugh A., 2001).

Fish blood is highly susceptible to both internal and external environment fluctuations, because pollutants are mainly transferred in the body through blood. Hematocrit values, haemoglobin content, number of red blood cells, white blood cells and haematological indices such as mean corpuscular volume", mean corpuscular haemoglobin" and „mean corpuscular haemoglobin concentrations" are the indicators of toxicity with wide potential for use in environmental monitoring and toxicity studies and are used to assess the functional status of the oxygen carrying capacity of the blood . (Sancho *E* .,2000 ; Barcellos L J. G ., 2003). These blood parameters have been considered as diagnostic indices of pathological condition and are important for the assessment of systemic functions and overall health of animals. In fish the status of nutrition, health, diseases and stress in response to altering environment can be assessed by fish haemotological parameters which serves as an essential tool for the biologists to understand the physiological and biochemical functions .

Objectives of the study :

1) To understand the varying effects of synthetic pyrethroids Cyfluthrin and fenvalarate on the biochemical constituents of *Gambusia* .
2) To understand the effect of synthetic pyrethroids Cyfluthrin and fenvalarate on on mosquito eating activity (behaviour)of *Gambusia,* which is used to control the population of mosquitoes.
3) To estimate the total protein, total free amino acids , nucleic acids (DNA and RNA) certain enzymes like LDH, SDH, AChE in the fish subjected to Cyfluthrin and fenvelarate

4) To study the total protein, total free amino acids , nucleic acids (DNA and RNA) certain enzymes like LDH, SDH, AChE in the control fish.
5) To study understand the short term and long term effects of synthetic pyrethroids Cyfluthrin and Fenvalerate on *Gambusia*.
6) To make a survey and collection of up to date literature on effect of synthetic pyrethroids on biochemical constituents fishes.
7) To explain the farmers the usage of minimal and appropriate toxicity synthetic pyrethroids , so that farmers can show some Consciousness while using the pesticides.

CHAPTER - 2

MATERIAL AND METHODS

Gambusia affinis

2. MATERIALS AND METHODS

Gambusia affinis (Baird and Girard, 1853) was introduced into India about 40 years ago from Italy and Thailand a native of coastal waters of United States from New Jersey southwards. Gambusia typically feeds on zooplankton, beetles, mayflies, caddis flies, mites and other invertebrates; mosquito larvae make up only a small portion of their diet. This is a fish used for the biological control of mosquito larvae in fresh water. The classification of the fish is as follows.

Order : Cyprinodontiformes
Family : Poeciliidae
Genus : *Gambusia*
Species : *affinis*

The main advantages of *Gambusia affinis* as an experimental animal in this present investigation are**:**

1) The fish is mostly used in many experiment tests as it easily available and is a very hardy fish can acclimatize to the laboratory conditions.
2) It is omnivorous and shows resistance to diseases

2.1 .COLLECTION, MAINTENANCE AND ACCLIMATIZATION OF EXPERIMENTAL FISH

Adult fresh water teleost fishes *Gambusia affinis* with weight (0.7-1.5 gm), length (2.3 - 3.2 cm) from both sexes were selected randomly for the experimental studies were collected from water bodies and fisheries department of Kurnool district , Andhra Pradesh , India. The collected fish were transported in polythene bags filled with half water with least disturbance . They are kept in aquarium tanks containing well aerated de-

chlorinated water to acclimatize to the laboratory conditions, with physico-chemical characteristics : temperature 24±2°C, pH 7.1±0.2 at 24°C, dissolved oxygen 9.6±0.8 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as $CaCO_3$/L, phosphate 0.39±0.002 µg/L, salinity in different cement tanks for one week . To prevent fungal infection the fish tanks were washed with potassium permanganate solution. Dead fish were removed as soon as possible to avoid water fouling.

Fishes were fed daily on commercial fish food. The unused food is removed daily and water was renewed daily .Test fishes were screened critically for any signs of stress, disease, physical damage and mortality. Injured, severely, diseased fishes were discarded. The fishes were exposed to sublethal concentrations for treated and control period of 96 hours. A control group was maintained with identical environment. The toxicant water as well as normal water is renewed every day. The fish were sacrificed from both experimental and control groups on 96 hours.

2.2 . Selection of Pesticides

In the present investigation two synthetic pyrethroids were selected namely Cyfluthrin and Fenvalerate.

2.2.1. Cyfluthrin

As per the reports pyrethroids are extremely toxic to fish and to some beneficial aquatic arthropods like lobster and shrimp. Cyfluthrin first registered by EPA in 1987, cyfluthrin is found in both restricted use (RUP) and general use insecticides (US EPA, 1987). Cyfluthrin is clear liquid, dark amber in color, with an oily to pasty consistency, and has a faint aromatic solvent odor at room temperature. Cyfluthrin, cyano (4-fluoro-3- phenoxyphenyl) methyl-3-(2,2 dichloroethenyl) -2,2-dimethylcyclopropanecarboxylate) is a broad spectrum synthetic type II pyrethroid insecticide.

Cyfluthrin acts as contact and stomach poison it attacks the nervous system; a nonsystemic insecticide used to control chewing and sucking insects and also in public health situations (Adams et al. 2002). Insects when in come

in contact with cyfluthrin die of starvation and desiccation, ceasing to feed. It is extensively used in agricultural crops, stored products, public health situations (i.e. cockroaches, mosquitoes, and flies), ornamentals, turf, and domestic pests. Insects like cockroaches, ants, grain beetles, silverfish, , fleas, flies, European corn borer, Colorado potato beetle, and many others are the main target of cyfluthrin use. (Farm Chemical Handbook, 1993; EXTOXNET, 2001; Hamman and Fuchs, 1981). Cyfluthrin act on non target organisms. The toxicity of cyfluthrin observed in various fish species is 0.68mg/L inrainbow trout (*Oncorhyncus mykiss*), 1.5mg/L in bluegill (*Lepomis macrochirus*), 22mg/L in carp (Cyprinus carpio), 3.2mg/L in golden orfe (*Leuciscus idus*), channel catfish (*Ictalurus punctatus*) and sheepshead minnow (*Cyprinodon variegatus*), 25.82mg/L for 48h (Bradbury and Coats, 1989) and 21.07mg/L for 72 h to Nile tilapia fry (*Oreochromis niloticus* L. 1758) (Benli, 2005). Cyfluthrin accumulation in the flesh of *Tilapia nilotica*, exposed to a sublethal dose of 0.001 mg/L, was 0.009 mg/L after 5 days of application (Al-Makkawy and Madbouly, 1999). Bradbury and Coats (1989) and Wolansky and Harrill (2008) also reviewed pyrethroid toxicology in mammals, birds, amphibians and both terrestrial and aquatic invertebrates.

Cyfluthrin causes oxidative damage in aquatic organisms resulting in the damage of essential cell components like DNA, protein, lipids and lipoproteins, caused by free oxygen radicals (Livingstone, 2001). Thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes (Kim., 2000). The products which has cyfluthrin are classified as acute Toxicity Category II (bearing the signal word "Warning") or Toxicity Category I (bearing the signal word "Danger") by EPA based on its potential ability to cause eye damage (Meister 1995).

2.2.2 . Characteristics of Cyfluthrin

Cyfluthrin stable at room temperature, but sensitive to sunlight (USEPA, 1992),

CAS Registry Number: 68359-37-5

Chemical Class: Synthetic pyrethroid

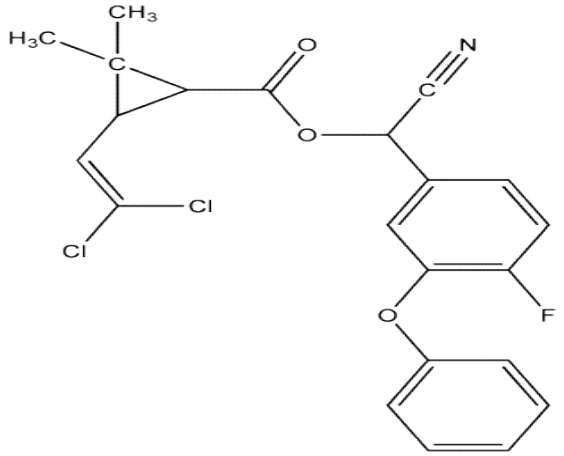
Use: Broad spectrum insecticide for a variety of agricultural crops

Toxicity rating: Moderately toxic

Signal Words: Caution. Warning. Danger

Health Effects: it acts as a neurotoxic poison, causes organ inflammation and induces skin paresthesia as per the animal studies.

Environmental Effects: Cyfluthrin is extremely toxic to bees, fish and other aquatic organisms.(source : Cyfluthrin *chemical watch* Factsheet, August 2007)



Cyfluthrin

cyano (4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate

2.2.3. Fenvalerate

Fenvalerate, is a clear viscious yellow liquid with a mild odor, a synthetic pyrethroid. First developed in 1974 by Sumitomo Chemical Co. of Japan (Sumicidin™), is most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. The biology of the non target species is affected by the use of insecticide along with pests. (Veeraiah and Durga Prasad, 1998; Anita Susan., 1999; Tilak., 2003; Tripathi and Verma, 2004; Sakr 2005; Babuvelmurugan 2007; Ramaneswari and Rao, 2008; Majumdar and Gupta, 2009).

The LC_{50} for 48 h of fenvalerate exposure to carp, *C. carpio* (8 cm) at p H 8-8.88 was reported to be 0.0210 mgL-1 (Jagan., 1989). Acute toxicity (LC_{50}) calculated over 96h for catla fingerlings (2.02 ± 0.1 g) was observed to be 6 µgL-1 (Tandon et al., 2005). LC_{50} value of fenvalerate at 96 hours to *Channa punctatus* (20 ± 3 g) is reported to be 2.13 µl L-1 (Singh S.P., 2007). Fish have a poor ability to metabolise and excrete fenvalerate (Bradbury., 1985) and thus are susceptible to even minute concentration of the pesticide.

In India, the pesticide is used primarily to control pests of cotton and vegetables (Madan ., 2000). Fenvalerate have half life of approximately two to four weeks in mineral and organic soils (Chapman and Harris, 1981). Deltamethrin is more persistent with a half-life of more than two months in soils (Chapman, 1981).

Fenvalerate is recently developed synthetic pyrethroids, which is highly toxic to fish but shows very low avian and mammalian toxicity. (Miyamoto, 1976; Casida., 1983). Fenvalerate has low persistence , broad spectrum of action on plant pests and metabolites/degradation products non toxic to man and other vertebrates. However, it is highly toxic to fish causing severe alterations in the metabolism of the organism, sometimes proving to be fatal if the concentrations are high**.**

Fenvalerate a synthetic pyrethroid is an effective against the bollworms ,aphids , weevils , leaf hoppers ,stem pod ,fruit borers etc which are common pests of agriculture.the technical grade containing 92% fenvalerate and 8% related compounds was obtained from saerle india ltd, Mumbai.

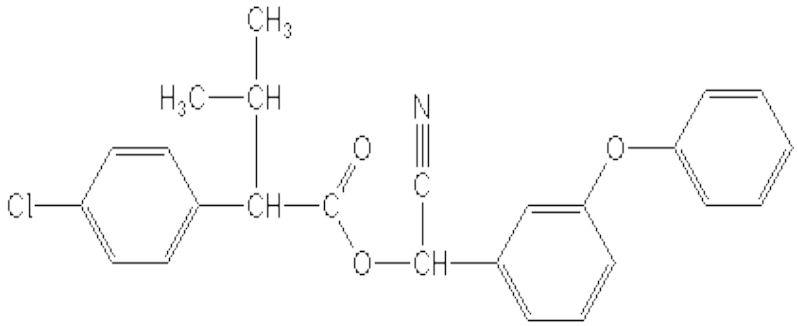
Fenvalerate

Generic name (IUPAC name) : (RS)-*alpha*-Cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate

Common name	:	Fenvalerate
Trade names	:	Pydrin, Sumicidin, Belmark
CAS number	:	51630-58-1

EPA Code : 109301

Year of initial registration : 1978



IUPAC name : (RS)-a-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate

Chemical Abstracts name : cyano (3-phenoxyphenyl)methyl 4-chloro-a-(1-methylethyl) benzene acetate

2.3. Toxicity Studies

To measure the impact of the toxicity of the selected pesticides acute toxicity tests were conducted. *Gambusia affinis* were periodically exposed to the concentrations of the same composition , usually once in every 24 hours by transferring the animals from one test chamber to another by adopting renewal technique of acute static test (Committee on methods for toxicity test with aquatic organisms , 1975) .The LC_{50} statistical estimation of the concentration of toxic material in water that killed 50 percent of the test animals under experimental conditions with specific time intervals (Spragu, 1971). This value was ideally suited for toxicity studies as it gave more acceptable and reproducible concentration required to affect 50 percent of the organic than any other value (Pickering and Henderson ,1966).

2.4 Experimental design

Group 1 : Fish exposed to tap water observed for 24 to 96 Hours (Untreated control).

Group 2 : Fish exposed to sublethal concentration of cyfluthrin of 24 to 96 hours.

Group 3 : Fish exposed to sublethal concentration of fenvalerate of 24 to 96 hours.

2. 5. Exploratory test

To avoid delay ,expense ,to save time and effoer exploratory test were conducted .The exploratory bioassay detyermines the approximate range of concentration of cyfluthrin and fenvalerate,which should be covered in the were exposed in plastic trough containing 10 litres of water. The following concentrations 80, 100, 120 , 140 ,160 ,180 ,200 ,220 ,240 ,260 ,280 ,300 ,320 ,340 ,360 ,and 380 ug/l were used in the present study . The preliminary tests were conducted to assess the highest concentration at which all fish survived for 96 hours and likewise the lowest concentrations at which most of the fish died simultaneously . Therefore, concentrations of cyfluthrin and fenvalerate falling within 100ug were prepared.

2.6. Definite tests

The trough was covered with nylon net to prevent the escape of the fish during the experimentation , to prevent the accumulation of excretory waste and to maintain the optimum dissolved oxygen content the test concentration was renewed every 24 hours with least disturbance .Control experiments were carriedout simultaneously .The

rate of mortality observed at 3, 6, 12, 24, 48,and 96 hours,the fishes considered as dead when they donot show respiratory movementand response to tactile stimuli were removed immediately. The concentration at which 100 percent mortality was observed within 24 hours was considered as lethal concentration(24 hours LC_{100}) and 100 percent survival observed upto 96 hours LC_{50} as used sublethal concentration (LC_0)

Calculation of LC_{50}

The LC_{50} values were obtained by probit analysis method based percentage of test animals surviving at concentrations that were lethal to more than half and less than half of the test subjects (Finney, 1971).

2.7. Acute toxicity studies

The toxicity studies will conducted by using the technical grade formulation available formulation of Fenvalerate and Cyfluthrin. Solutions of desired concentrations were prepared in 95% acetone to get the stock solution as well as working solution as 100mg/100ml and 1mg/1ml of toxicant chemical Fenvalarate and Cyfluthrin in both technical formulation use of acetone in control as recommended by EPA will be followed**.**

The the acclimatized fishes were kept in cement tanks and ten fishes in two groups control fishes will be kept in dechlorinated water only. Each set of experiment was replicated six times. Mortality was recorded every 24h during the observation period of 96h.

The LC_{50} values were calculated for *Gambusia affinis* exposed to of cyfluthrin and fenvalerate at 3, 6,12,24, 48, 72 and 96 hours and they were found to be 36.12, 34.41, 33.76, 31.32, 28.97, 26.69, 24.73 **µg/L –** cyfluthrin and 19.67, 18.98, 17.29, 16.98, 15.93, 14.75, 13.87 **µg/L -** fenvalerate respectively (Table -1) .The toxicity curve (Fig-1) showed the mode of action and the toxicity of cyfluthrin and fenvalerate for different concentrations at different periods. A similar result with regard to fenvalerte toxicity in

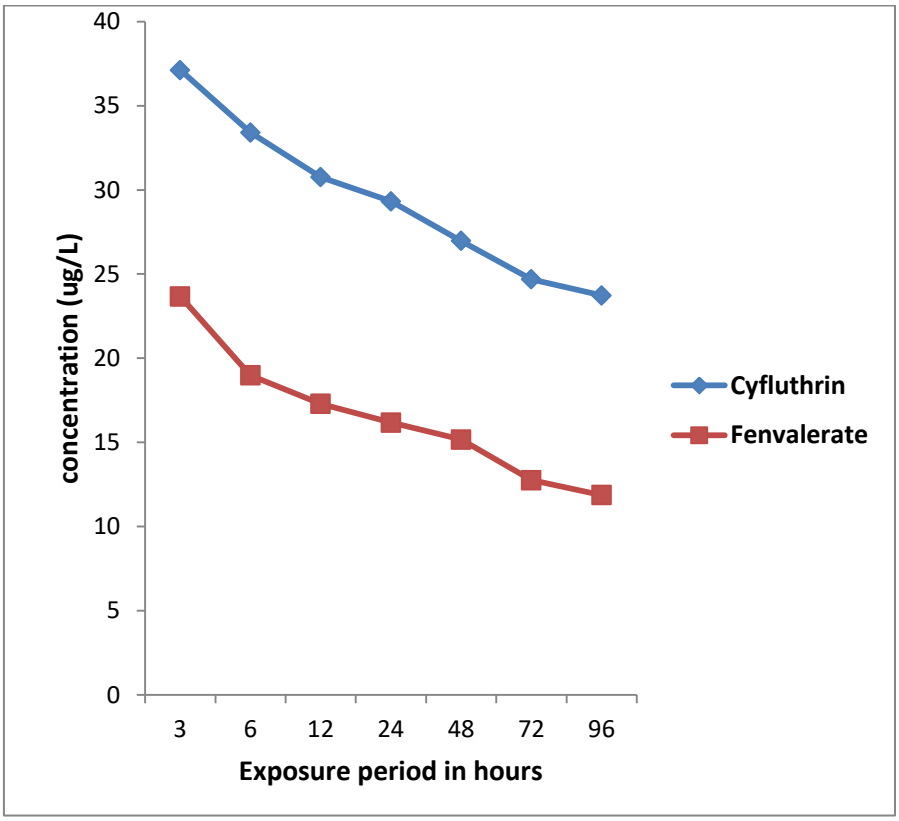
Gambussia affinis , the LC_{50} value of 15.0 **µg** /L (48 h) was reported . (Smith and Stratton ., 1986)

In the present study, it was observed that the mortality rate increased with the higher concentration of cyfluthrin and fenvalerate over a short period of time and diminished with decreasing concentration during the subsequent hours of treatment.

Table 1. Median lethal concentration LC_{50} values for the fish *Gambusia affinis* exposed to different concentrations of cyfluthrin and fenvalerate for different at different periods.

S.No	Hours	LC_{50} **µg/L -** cyfluthrin	LC_{50} **µg/L -** fenvalerate
1	3	37.12	23.67
2	6	33.41	18.98
3	12	30.76	17.29
4	24	29.32	16.18
5	48	26.97	14.97
6	72	24.69	12.75
7	96	23.73	11.87

Fig 1. Median lethal concentration LC_{50} values for the fish *Gambusia affinis* exposed to different concentrations of cyfluthrin and fenvalerate for different at different periods



2.8. Statistical analysis

The data obtained from the quantitative study were expressed as the mean ± S.E. The mean values were calculated from 6 individual observations. P— values were calculated by the students 't' test.The two mean values obtained were considered significant from each other with standard error.

2.9. Haematological Studies

2.9.1. Red blood corpuscle (RBC) count:

RBC count was made with a Neubauer Crystalling Counting chamber as described by Davidson and Henry (1969). Blood was collected in a vital containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant.

The blood was drawn from the vial into the RBC pipette upto 0.5 mark and immediately the diluting fluid is drawn upto the mark 101 (thus the dilution is 1:200). The solution was mixed thoroughly by shaking gently. It was allowed to stand for 2 to 3 minutes. The counting chamber and cover slip were cleaned and the cover slip was placed over the ruled area.

Again the solution was mixed gently and the sternful of solution was expelled and a drop of fluid was allowed to flow under the coverslip, holding the pipette at an angle 40°. It was allowed to stand still (2 or 3 min) till the RBC settles. Afterwards the ruled counting area was focused under the microscope and the number of RBCs was counted in five small squares of the RBC column under high power and the numbers of RBCs per sq.mm were calculated accordingly. (Shodhganga.inflibnet.ac.in)

$$\frac{\text{No. of cells x Dilution factor x Depth factor}}{\text{Area counted}}$$

2.9.2 . Estimation of Haemoglobin (Hb) concentration :

The haemoglobin concentration was estimated by acid heamatin method (Sahli, 1966). N/10 hydrochloric acid (HC1) was taken upto 10.0 mark in the graduated tube. Blood was sucked into the Hb pipette upto the mark and was transferred into the graduated tube containing N/10 hydrochloric acid. It was allowed to stand in dark for 10 to 20 minutes after thorough mixing. Now N/10 HC1 was added drop by drop, mixing between each addition, until the blood colour matched with the standard colour. And then the results were read from the scale on the graduated tube and the Hb concentration is expressed in grams percent. (Shodhganga.inflibnet.ac.in)

2.9.3 . Estimation of packed cell volume (PCV) :

PCV was estimated by microhaematocrit method (Sachalm , 1975). The blood was drawn into capillary tubes containing anticoagulant by capillary suction to 3A of their length. The outside of the capillary tubes were wiped free of blood and the index finger was placed over the moist ends to hold the column of the blood in place because the opposite dry ends were forced into the sealing material to form a tight plug. The capillary tubes were placed in the centrifuge with the sealed ends pointing outward and centrifuged at 12000 rpm for 2 min.

PCV was determined by rolling the capillary tubes over a reader card until the top of the plasma column was aligned with the 100% line and the bottom of the packed erythrocytes was on the zero line. The line that crossed the top of the packed erythrocyte column represented the PCV in percent. (Shodhganga.inflibnet.ac.in)

2.9.4 .Estimation of Blood Glucose

Glucose was determined by colorimetric method of Nelson and Somogyi (1952). To 0.1 ml of blood, 3.9 ml of deproteinizing solution (5% zinc sulphate and 0.3 N sodium hydroxide solution in 1:1 ratio) was added. The mixture was centrifuged at 3000 rpm for 10 minutes. To 1 ml of the supernatant 1 ml of alkaline copper reagent was added. The mixture was shaken vigorously and heated in a boiling water bath exactly for 20 minutes. The mixture was cooled and added 1 ml of arsenomolybdate colour reagent. Made the entire solution to 10 ml with distilled water and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 540 nm. A blank and glucose standards were additionally run similarly. Glucose levels in the blood are expressed as mg % (Shodhganga.inflibnet.ac.in)

2.10. Tissue Biochemical Studies

2.10.1. Estimation of tissue protein

Protein in the tissues was determined after trichloro acetic precipitation by the method of Lowry et al.1951.

Reagents

Alkaline copper reagent ,reagent A : 2% of sodium carbonate in 0.1 N NAOH, Reagent B: 0.5% copper sulphate in 1% sodium potassium tartarate, Reagent C; 50 ml reagent A was mixed with 0.5ml of reagent B just before use. Folins phenol reagent : Dilute 1:2 with distilled water .Stock standard : 100mg of bovine serum albumin /100ml of water and working standard : 10ml of the stock standard was diluted to 100ml to get a working standard containing 0.1mg/ml.

Procedure

0.5 ml of the supernatant was mixed with 0.5 ml of 10% TCA and centrifuged for 10 minutes. The precipitate was dissolved in 1.0ml of 0.1N NAOH .From this an aliquot was taken and 4.5 ml of alkaline copper reagent was added and allowed to stand at room temperature for 10 minutes .0.5 ml of Folins phenol reagent was added and the blue colour developed was read after 20 minutes . A standard curve is obtained with standard bovine albumin and was to assy the protein level for enzyme activity. Values are expressed as mg/g wet weight of the tissues.

2.10.2. Estimation of tissue total free amino acid in tissues

Total free amino acids content of the respective tissues was determined by adopting of Moore and Stein (1954).

Reagents

10% Trichloacetic acid : 10 g in 100ml of distilled water , Ninhydrin reagent, 2g of citric acid in 20ml of in NAOH upto 50ml by adding distilled water and 80mg stannous chloride , 2g of Ninhydrine dissolved in 50ml of acetone and mix A and B in equal volume

Procedure

The tissues (kidney, gill, liver) were isolated on ice quickly weighed in a balance in a cold room and separately homogenated in cold 10% TCA solution (0.1ml homogenate contains 10 mg of each tisuue) and then centrifuged at 3000 rpm for 15 minutes .From the contents clear supernatant 0.1 ml was taken in a claen test tube and then 0.9 ml of 10% TCA and 20ml of ninhydrin reagent was added and then kept in a boiling water bath for 6.5 minutes accurately .The mixure was cooled immediately in tap water and the colour was read at 570nm against reagent black in the UV Spectrophotometer. Tyrosine was used to construct the standard graph and the values are expressed in ug/gm wet weight of the tissue.

2.10.3. Estimation of tissue Glucose

Glucose in the organs was determined by colorimetric method as described by Nelson and Somogyi (1952). 5% homogenates of the organs were prepared in pure distilled water. To 0.5 ml of homogenate, 4.5 ml of 10% TCA solution was added and the mixture was centrifuged at 3000 rpm for 10 minutes. To 1 ml of the supernatant, 1 ml of alkaline copper reagent was added, shaken vigorously and heated in a boiling water bath exactly for 20 minutes. After cooling it, 1 ml of colour reagent arsenomolybdate was added. The entire solution was made to 10 ml with distilled water and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 540 nm. A blank and glucose standards were run addditionaly. The glucose content is expressed as mg/gm wet weight of the organ. (Shodhganga.inflibnet.ac.in)

2.11. Enzymological Studies

2.11.1. Estimation of Succinate dehydrogenase (SDH) activity

The tissue was isolated from the fish in the cold room and 5% homogenate was prepared in 0.25 m sucrose solution and centrifuged at 2500 rpm for 15 minutes to remove all debris .The supernatant was used for the enzyme assay. Succinate dehydrogenase activity in the organs was estimated using the colorimetric method of Nachlas *et al,* (1960). A 5% homogenate (W/V) was prepared in 0.24 M ice cold sucrose solution centrifuged at 3000 rpm for 10 minutes and the supernatant was taken as the source of enzyme. The incubation mixture consisted of 0.2 ml of 0.4 M phosphate buffer (pH 7.7), 0.2 ml of 0.2 M sodium succinate, 1 ml of 0.004 M 2-(P-indophenol)-3Pnitrophenyl- tetrazolium chloride (INT), 0.1 ml of 0.005 M phenazine methosulphate and 0.5 ml of 5% enzyme preparation. The mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 6 ml of glacial acetic acid.

The formozan formed was extracted into 6 ml of toluene overnight at 0°C and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 495 nm.

A blank taking 0.5 ml of distilled water and control taking 0.5 ml of boiled enzyme was also run for comparison. INT standards were prepared alongside for comparison. The activity is expressed as p M formozan/mg protein/h.(Shodhganga.inflibnet.ac.in)

2.11.2. Estimation of Lactate dehydrogenase activity

Lactate dehydrogenase activity in selected tissues was assayed by the method of Srikanthan and krishnamoorthy (1955) as modified by Govindappa and Swami 1965. A 5% homogenate (W/V) was prepared in 0.25 M ice cold sucrose solution, centrifuged at 2500 rpm for 15 minutes and

the supernatant was taken as the source of enzyme. The incubation mixture consisted of 1 ml of 0.04 M phosphate buffer (pH 7.4), 0.5 ml of 0.1 M lithium lactate, 1 ml of 0.0001 M nicotinamide adenine dinucleotide (NAD), 1 ml of 0.004 M 2-(Pindophenol)-3 P-nitrophenyl-5-phenyl tetrazolium chloride (INT) and 0.5 ml of 5% enzyme preparation. The mixture was incubated at 37°C for 30 minutes and then added 6 ml of glacial acetic acid to stop the reaction. The formozan formed was extracted into 6 ml of toluene over night at 0°C. The optical density of the colour developed was measured in a spectrophotometer at a wavelength of495 nm. A blank using 0.5 ml of distilled water and a control by taking 0.5 ml of boiled enzyme was also run similarly. INT standards were prepared along side for comparison. The enzyme activity is expressed as p M formozan/mg protein/h. (Shodhganga.inflibnet.ac.in).

2.11.3. Estimation of Glucose 6-phosphate

Glucose-6-phosphatase activity in the organs was estimated using the method of Yeung *et al,* (1968). A 5% homogenate (W/V) was prepared in 0.25 M ice cold sucrose solution. 0.5 ml of 0.2 M tris malate buffer, 0.2 ml of 0.05 M glucose-6-phosphate and 0.2 ml of distilled water were incubated at 37°C for 5 minutes and to this 0.1 ml of homogenate was added and incubated exactly for 10 minutes. With the addition of adding 1 ml of 10% trichloroacetic acid the reaction was stopped. A blank was also run similarly. Finally, the inorganic phosphates liberated were estimated by the method of Fiske and Subba Rao (1925) at a wavelength of 660 nm and the activity is expressed as ug Pi liberated/mg protein/h. (Shodhganga.inflibnet.ac.in)

2.11.4. Estimation of Acetylcholinesterase activity (AChE) activity

The method of AChE activity estimation is popularly known as Ellman's method named after George Ellman who developed this method in 1961 The esterase activity is measured by providing an artificial substrate, acetylthiocholinelodide (ACI). Thiocholine released because of the cleavage of

ACI by AChE is allowed to react with the -SH reagent 5,5'- dithiobis- (2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm. Assay was performed by adding 2.9ml of O.lmM sodium phosphate buffer (p.H-8) to 50ml of supernatant of different region of brain and then incubated at 37 for 5 minutes.

After incubation 40ul of Acetylthiocholine iodide (154.38mM), 10ul of DTNB (10mM) was added, mixed by inverting cuvette and absorbance was taken at 412nm for 150 sec at interval of 30sec using UV spectrophotometer. The extinction coefficient of the thionitrobenzoic acid is 1.36 x 104/ molar/ centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is than taken as direct estimate of AChE activity. (Shodhganga.inflibnet.ac.in)

2.12. Estimation of Nucleic acids

Nucleic acids were extracted from the tissues by the method described by Schneider (1957).

Principle: Nucleic acids are separated from the other tissue components by exploiting their property of preferential solubility in hot trichloroacetic acid (TCA) solution.

Procedure: Known weight of tissue was rinsed finely and homogenized in distilled water, to homogenate 5 ml of 10% ice cold TCA was added and was kept in an ice bath for 30 minutes to allow complete precipitation of proteins and nucleic acids. The mixture was centrifuged and the precipitate was later treated with absolute alcohol and centrifuged to remove lipid materials.

The precipitate free of lipids was suspended in required amount of 5% TCA and placed in a water bath maintained at 90°C for 15 minutes with occasional stirring which facilitates the quantitative separation of nucleic acids from the precipitated proteins. This was centrifuged and the supernatant was used for the estimation of Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA). (Shodhganga.inflibnet.ac.in)

2.12.1. Estimation of DNA

Principle: Estimation of DNA was performed by using diphenylamine reagent (Schneider, 1957). This method is based on the property of deoxyribose moiety of DNA to form hydroxy laevulaldehyde in TCA solution. This reacts with diphenylamine to give a coloured complex. The intensity of colouration is presumed to be proportional to the pentose (deoxyribose) concentration in the DNA hydrolyzate. Total DNA content was expressed as mg/100g wet tissue.

Reagents A. Dische's diphenylamine reagent 1 g of diphenylamine was dissolved in 100 ml of glacial acetic acid. After adding 2.75 ml of Conc. H2SO4, the solution was mixed and stored in dark. B. Standard DNA Solution Freshly prepared by dissolving 100 mg of commercially available DNA with 100 ml of distilled water.

Procedure: 1ml of isolated nucleic acid TCA hydrolyzate was taken and mixed well with 0.5 ml of perchloric acid. The solution was kept for boiling after adding 2 ml of reagent A. Boiling was done in water bath for 10 minutes. The solution was then cooled under tap water and optical density was measured at 565 nm against blank. (Shodhganga.inflibnet.ac.in)

2.12.2. Estimation of RNA

Principle : Total RNA content was estimated by the methods of Schneider (1957) using orcinol reagent. The ribose moiety of RNA in the TCA extract is converted to furfural which condenses with orcinol to yield a blue coloured complex. The colour intensity is presumed to be proportional to RNA concentration in the extract. Total RNA content was expressed as mg/100g wet tissue.

Reagents A. Orcinol reagent 500 ml of 30 % HCl was taken and 1 g of orcinol was dissolved well in it. 4-5 ml of 10 % ferric chloride was added and mixed in it. The solution was stored in a brown bottle. B. Standard RNA Solution dissolved 100 mg of RNA in 100 ml of distilled water

Procedure: 2 ml of nucleic acid TCA extract was taken and added with 2 ml of orcinol reagent. The solution was mixed and kept for boiling for 20 minutes and cooled thereafter. Absorbance was read at 670 nm in UV-spectrophotometer. (Shodhganga.inflibnet.ac.in).

Significance of the present study

Gambusia affinis is a native of coastal waters of United States from New Jersey southwards, introduced into India from Italy and Thailand. *Gambusia affinis* has been in use in India since 1928. It is a very hardy fish and can adapt to wide variations in temperature as well as to chemical and organic content of the water but does not tolerate very high organic pollution. This is a common larvacious fish used for the biological control of mosquito larvae in fresh water. *Gambusia* typically eat zooplankton, beetles, mayflies, caddis flies, mites and other invertebrates; mosquito larvae make up only a small portion of their diet. Fish have been widely used in public health, since as early as 1903. One of the most successful and widely used biological control agent against mosquito larvae is the top water minnow or mosquito fish *Gambusia affinis*. *G. affinis* is commonly present in the pond and streams near agricultural areas and also it is easy to maintain this species in laboratory (Öztürk and Ikiz, 2003, Güner, 2009).Fish other than *Gambusia* which has received the most attention as a mosquito control agent is *Poecilia reticulata*, the common guppy. So, the aim of this study was to examine the toxicity of Cyfluthrin and Fenvalerate to the freshwater teleost fish *Gambusia affinis* and evaluate the effect of sub-lethal doses of this pesticides on haemotology , biochemical and enzymological and aspects of the fish.

CHAPTER - 3

RESULTS

3.1. HAEMATOLOGICAL PARMETERS

The quantitative changes in haematological parameters like RBC, WBC,Hb and PCV of blood cells in the fresh water fish *Gambusia affinis* fish in all groups control (group-1) sub lethal concentrations of cyfluthrin (group-2) and fenvalerate (group-3) exposed to 24 to 96 hours are observed .

3.1.1. Red blood corpuscles (RBC)

In the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed the decrease in the RBC content in comparison with control group .The percentage changes with cyfluthrin are - 12.65, - 28 ,- 31.8, and - 40.3 for 24,48,72 and 96 hours respectively. Whereas The percentage changes with fenvalerate are - 13.77, - 24.7 , - 35.14 and - 46.28 for 24,48,72 and 96 hours respectively. The RBC content in the blood cells counts for 3 groups are statistically significant at 1% and 5% levels (table 2: fig.2)

3.1.2. White blood corpuscles (RBC)

There is a noticeable increase in the WBC content in fresh water fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) when compare to the control group. The percentage changes with cyfluthrin are +27.14 , + 48.93 , + 68.67, +74.42 for 24,48,72 and 96 hours respectively. Whereas The percentage changes with fenvalerate are + 30.56, +46.12, + 74.89 , and +83.28 for 24,48,72 and 96 hours respectively. The WBC content in the blood cells counts for 3 groups are recorded are statistically significant at 1% and 5% levels (table 2: fig.2)

3.1.3. Haemoglobin (Hb)

The haemoglobin content of the blood decreased in fish exposed to pesticides cyfluthrin (group-2) and fenvalerate (group-3) in comparison with the control group . The percentage changes with cyfluthrin are - 12.40, - 17.94, - 24.97, - 29.81 for 24,48,72 and 96 hours respectively. Whereas The percentage changes with fenvalerate are , -14 , - 19.55, 26.02 and -31.13 for 24,48,72 and 96 hours respectively.

The haemoglobin content in the blood cells counts for 3 groups are recorded are statistically significant at 1% and 5% levels (table 2: fig.2) .

3.1.4. Packed cell volume (PCV)

In the present study the level of PCV values of the blood decreased in *Gambusia affinis* exposed to pesticides cyfluthrin (group-2) and fenvalerate (group-3) in comparison with the control group. The percent changes are - 10.16, - 18.77 , - 25.29 , - 28.85 , for 24,48,72 and 96 hours respectively in group 2 (cyfluthrin) . whereas the percentage change in group 3 (fenvalerate) are -12 .29, - 20.4 , -26.43 , and -31.81 , for 24,48,72 and 96 hours respectively. The levels of PCV content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 2: fig.2)

3.1.5. Glucose

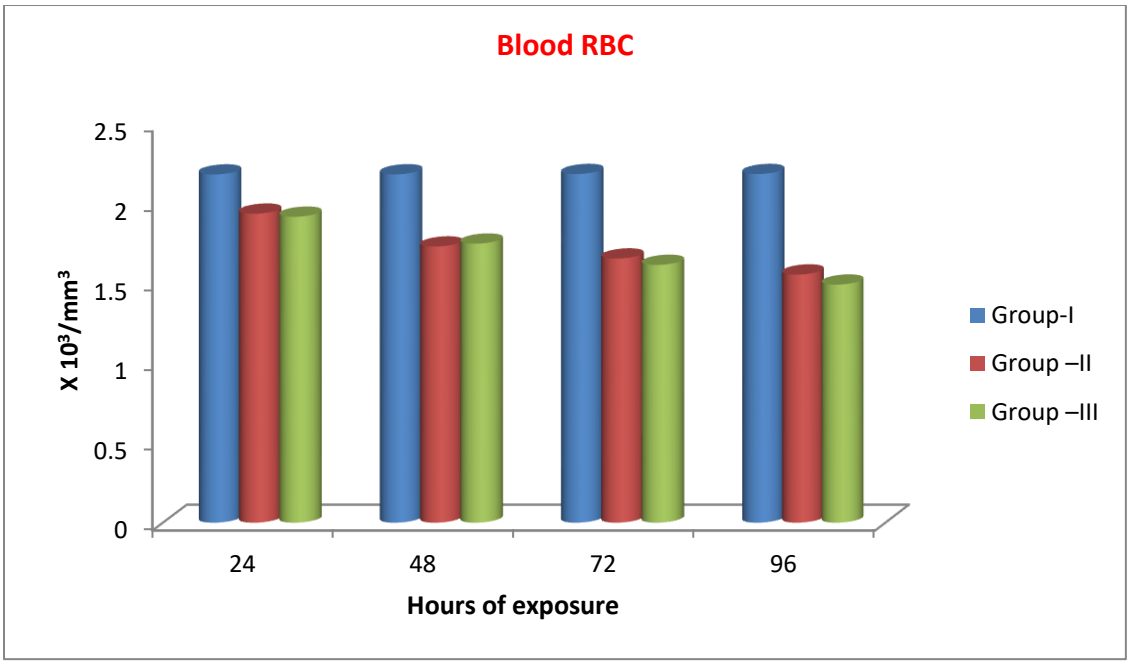
There is a significant increase in the content of blood glucose in the fresh water fish *Gambusia affinnis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with the control group. The percent changes in group 2 i.e cyfluthrin exposed group is + 6.80, + 11.27, + 13.46, +18.51 for 24,48,72 and 96 hours respectively. In case *Gambusia affinnis* exposed to fenvalerate the percent changes are + 7.74 , + 12.74, + 15.51 + 20.72 and for 24,48,72 and 96 hours respectively. The levels of glucose content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 2: fig.2).

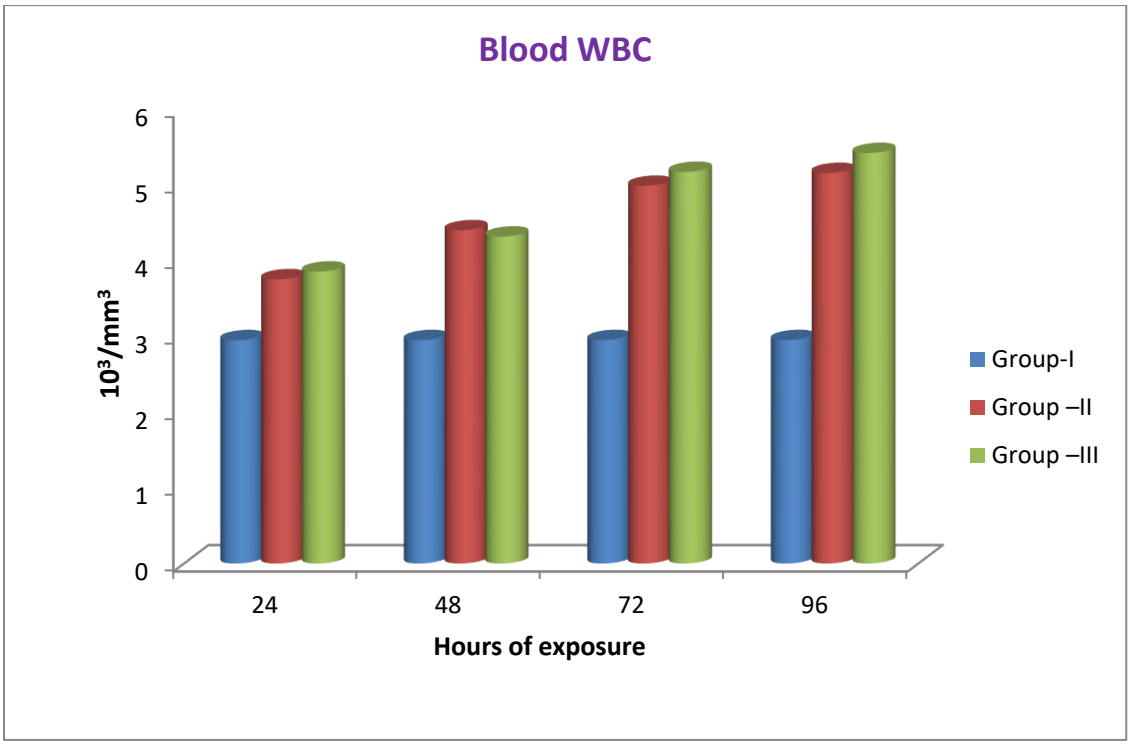
Table 2 : Variations of RBC (X 10^6/mm^3), WBC (X 10^3/mm^3, Haemoglobin (Hb) g/dL., and PCV (%) , Blood glucose (mg/dL),values values in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

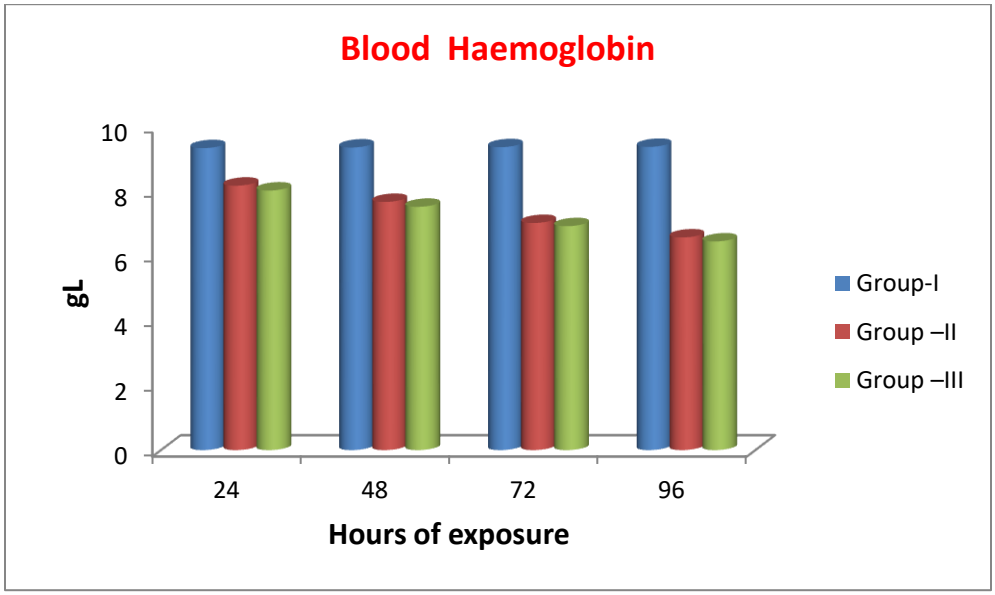
Blood	Groups	Hours of exposure			
		24	48	72	96
RBC	Group-I Control	2.181 ± 0.320	2.182 ± 0.331	2.184 ± 0.320	2.184 ± 0.322
	Group –II- Cyfluthrin % COC	1.936** ± 0.124 % - 12.65	1.731** ± 0.213 % - 28	1.656** ± 0.432 % - 31.8	1.556** ± 0.312 % - 40.3
	Group –III- Fenvalerate % COC	1.917** ± 0.421 % - 13.77	1.749** ± 0.322 % - 24.7	1.616** ± 0.365 % - 35.14	1.493** ± 0.419 % - 46.28
WBC	Group-I Control	2.954 ± 0.252	2.955 ± 0.271	2.956 ± 0.251	2.956 ± 0.253
	Group –II- Cyfluthrin % COC	3.756** ± 0.281 % +27.14	4.401** ± 0.632 % + 48.93	4.986** ± 0.675 % + 68.67	5.156** ± 0.341 % +74.42
	Group –III- Fenvalerate % COC	3.857** ± 0.813 % + 30.56	4.318** ± 0.324 % +46.12	5.170** ± 0.753 % + 74.89	5.418** ± 0.443 % +83.28
Haemoglobin	Group-I Control	9.32 ± 0.981	9.34 ± 0.983	9.35 ± 0.985	9.36 ± 0.987
	Group –II- Cyfluthrin % COC	8.164** ± 0.643 % - 12.40	7.664** ± 0.541 % - 17.94	7.015** ± 0.751 % - 24.97	6.569** ± 0.512 % - 29.81
	Group –III- Fenvalerate % COC	8.015** ± 0.321 % -14	7.514** ± 0.412 % - 19.55	6.917** ± 0.516 % -26.02	6.446** ± 0.422 % -31.13
PCV	Group-I Control	30.738 ± 0.632	30.739 ± 0.630	30.740 ± 0.632	30.740 ± 0.629
	Group –II- Cyfluthrin % COC	27.162** ± 0.346 % - 10.16	24.967**± 0.448 % - 18.77	22.964**± 0.532 % - 25.29	21.869** ± 0.521 % - 28.85
	Group –III- Fenvalerate % COC	26.958** ± 0.436 % -12 .29	24.465**± 0.344 % -20.4	22.613**± 0.421 % -26.43	20.961** ± 0.643 % -31.81
Glucose	Group-I Control	51.174 ± 0.561	51.175 ± 0.481	51.176 ± 0.808	51.176 ± 0.861
	Group –II- Cyfluthrin % COC	54.654* ± 0.786 % + 6.80	56.947** ± 0.867 % + 11.27	58.117** ± 0.572 % + 13.46	60.652** ± 0.886 % +18.51
	Group –III- Fenvalerate % COC	55.136* ± 0.675 % + 7.74	57.699** ± 0.671 % + 12.74	59.118** ± 0.722 % + 15.51	61.782** ± 0.681 % + 20.72

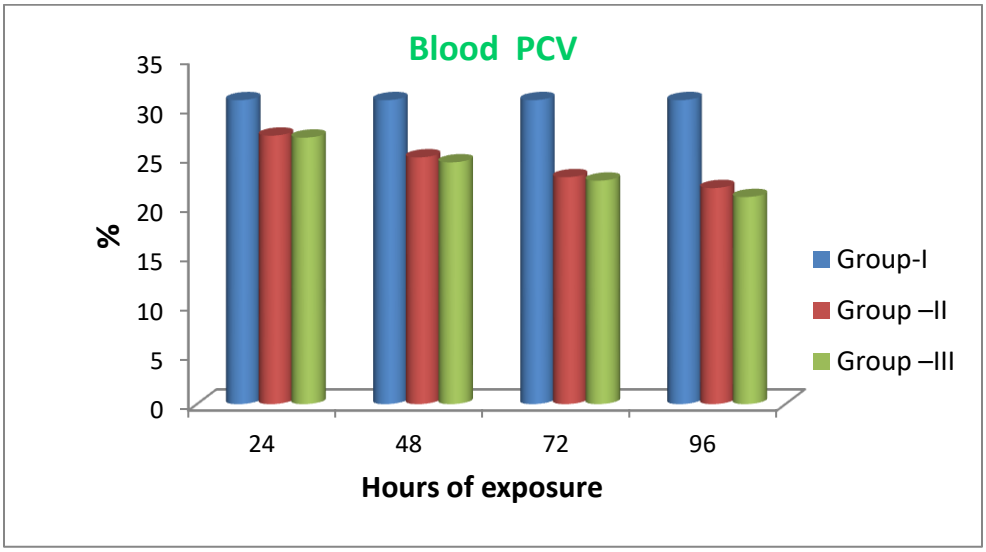
Values are mean ± S.E Mean of six individual observations and student t-test, significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased . %COC (hange over control) ;

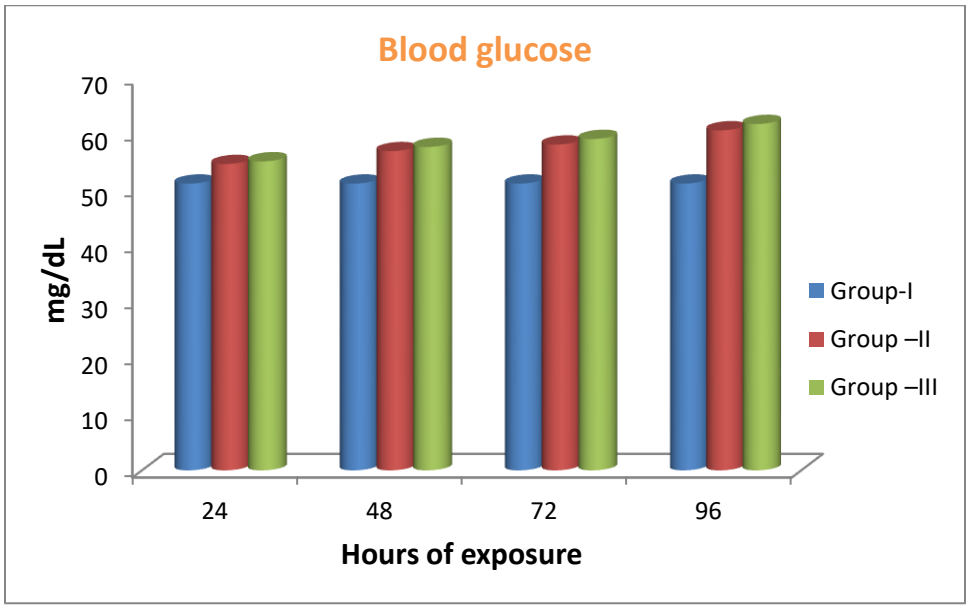
Fig. 2 Changes in the level of blood RBC (X $10^6/mm^3$), WBC (X $10^3/mm^3$, Haemoglobin (Hb) g/dL., and PCV (%) , Blood glucose (mg/dL),values values in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours.






Blood Haemoglobin
10
8
6
4
2
0
gL
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III


Blood PCV
35
30
25
20
15
10
5
0
%
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III


Blood glucose
70
60
50
40
30
20
10
0
mg/dL
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III

3.2. TISSUE BIOCHEMICAL PARAMETERS

The quantitative changes in biochemical parameters like proten, amino acid and glucose of the gill, liver, kidney and muscle tissues in the fresh water fish *Gambusia affinis* fish in all groups , control (group-1) sub lethal concentrations of cyfluthrin (group-2) and fenvalerate (group-3) exposed to 24 to 96 hours are observed.

3.2.1.Tissue protein

3.2.1.1. Gill

The protein content of the gill tissue decreased in fish *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3)when compare with the control group. The percent decrease with cyfluthrin (group-2) are - 9.45, - 17.68, - 22.30, - 33.34 for 24,48,72 and 96 hours respectively. While the *Gambusia affinis* exposed to fenvalerate (group-3) are - 10.39, - 16, - 24.12, - 36.47 for 24,48,72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 3: fig.3).

3.2.1.2 .Liver

The content of protein in liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline when compared with the control group. The percent decline with cyfluthrin (group-2) are - 4.48, - 8.99, - 12.50 , - 16.73, for 24, 48, 72 and 96 hours respectively. The percent decline with fenvalerate (group-3) are - 5.93 , - 10.50, - 15 , - 20.02 for 24, 48, 72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 3: fig.3).

3.2.1.3.Kidney

In the present study the protein content in the tissues of kidney was altered i.e., decreased in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of alterations are - 5.09, - 11.58 , - 17.59 , - 22.25 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 11.64, - 15.92, - 22.58 , - 28.82 25 for 24, 48, 72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 3: fig.3).

3.2.1.4. Muscle

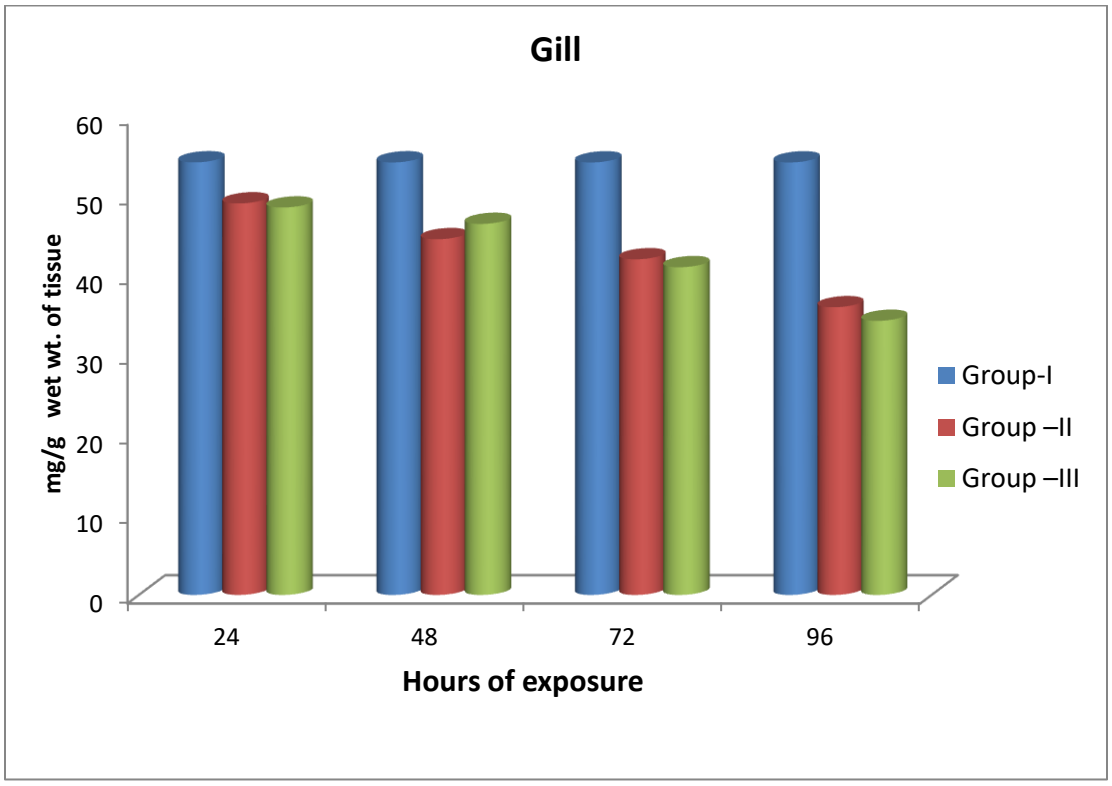
The levels of protein content in the muscle shown a declining trend in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of decline in fish exposed to cyfluthrin (group-2) are - 9.92 , - 18.37 , - 23.23 , - 29.91 for 24, 48, 72 and 96 hours respectively. The decline percentage in fish exposed to fenvalerate (group-3) are - 11.12 , - 19.80 , - 21.82 , - 31.55 for 24, 48, 72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (table 3: fig.3).

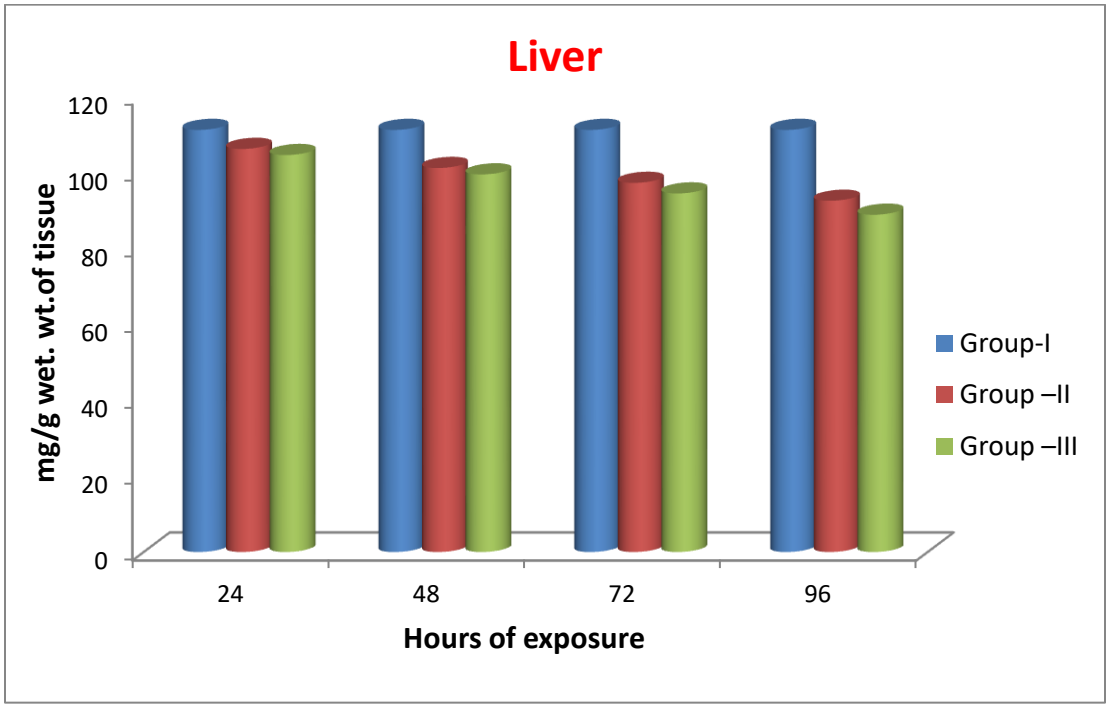
Table 3 : Variations of Proteins (mg /g wet wt. of tissue) content in the in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

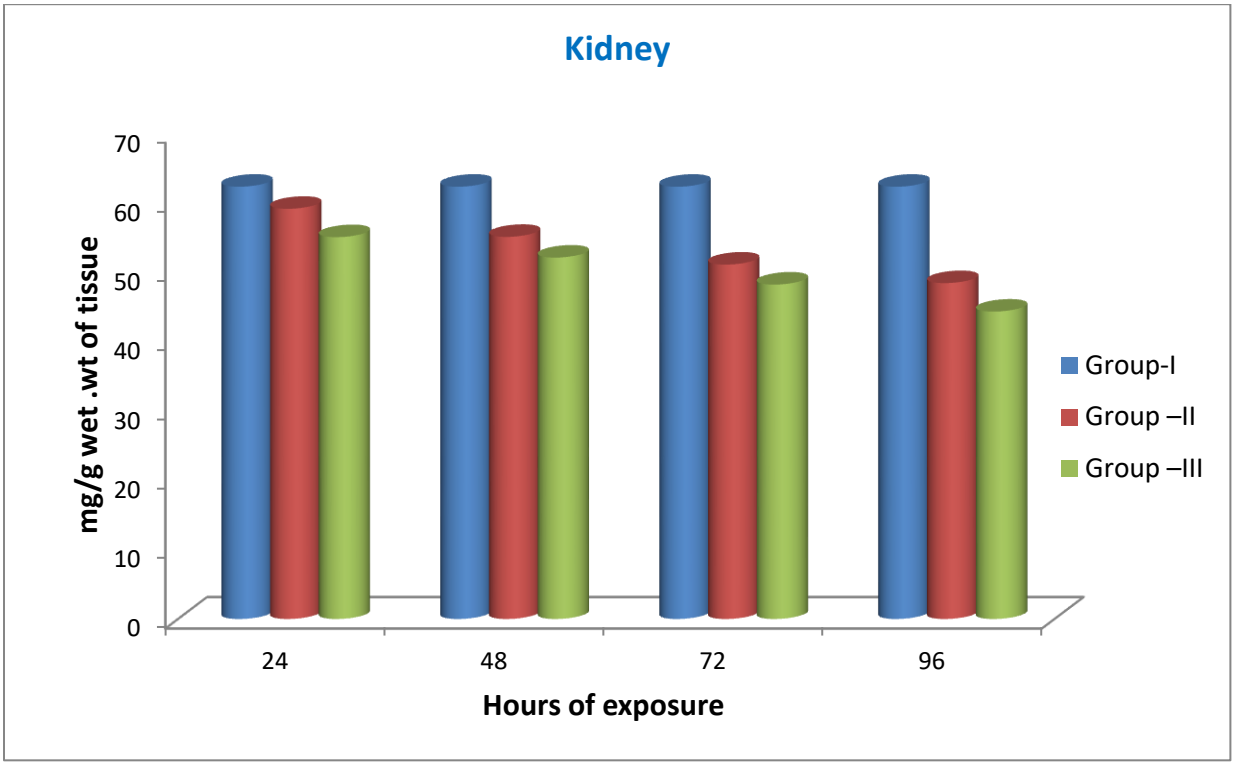
Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control 54	54.254 ± 0.821	54.250 ± 0.616	54.243 ± 0.643	54.245 ± 0.753
	Group –II- Cyfluthrin % COC	49.125** ± 0.613 % - 9.45	44.654** ± 0.710 % - 17.68	42.142**± 0.752 % - 22.30	36.156** ± 0.886 % - 33.34
	Group –III- Fenvalerate % COC	48.613* ± 0.610 % - 10.39	46.567** ± 0.720 % - 16	41.156**± 0.741 % - 24.12	34.457** ± 0.826 % - 36.47
Liver	Group-I Control	111.134 ± 0.621	111.133 ± 0.623	111.134 ± 0.625	111.135 ± 0.626
	Group –II- Cyfluthrin % COC	106.147*± 0.660 % - 4.48	101.142** ± 0.660 % - 8.99	97.241** ± 0.663 % - 12.50	92.546** ± 0.665 % - 16.73
	Group –III- Fenvalerate % COC	104.542*± 0.561 % - 5.93	99.458** ± 0.560 % - 10.50	94.465** ± 0.565 % - 15	88.879** ± 0.567 % - 20.02
Kidney	Group-I Control	62.343 ± 0.672	62.341 ± 0.675	62.335 ± 0.678	62.336 ± 0.670
	Group –II- Cyfluthrin % COC	59.164** ± 0.691 % - 5.09	55.126** ± 0.689 % - 11.58	51.1369** ± 0.690 % - 17.59	48.466** ± 0.691 % - 22.25
	Group –III- Fenvalerate % COC	55.084** ± 0.601 % - 11.64	52.1413** ± 0.603 % - 15.92	48.257** ± 0.606 % - 22.58	44.365** ± 0.610 % - 28.82
Muscle	Group-I Control	121.464 ± 0.612	121.463 ± 0.611	121.464 ± 0.613	121.465 ± 0.615
	Group –II- Cyfluthrin % COC	109.41**± 0.112 % - 9.92	99.145**± 0.114 % - 18.37	93. 245**± 0.115 % - 23.23	85.124**± 0.116 % - 29.91
	Group –III- Fenvalerate % COC	107.954**± 0.112 % - 11.12	97.412**± 0.114 % - 19.80	94. 954**± 0.115 % - 21.82	83.147**± 0.116 % - 31.55

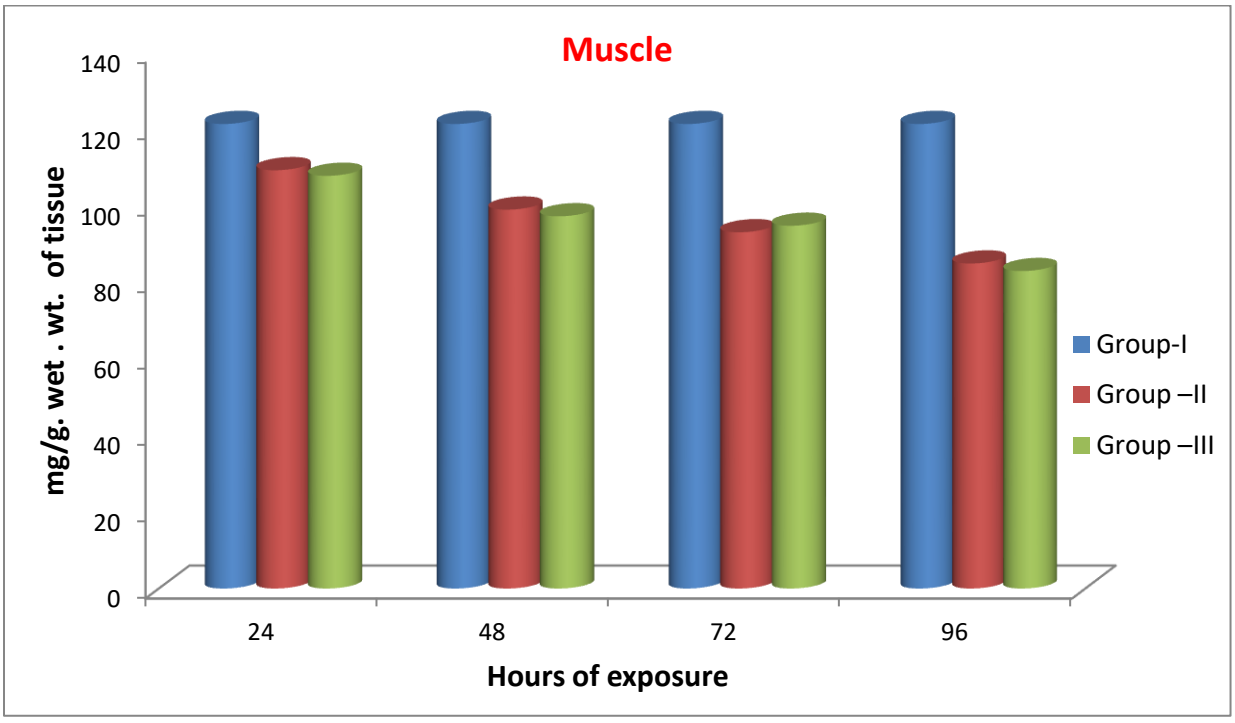
Values are mean **±** S.E Mean of six individual observations and student t-test. Significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 3 : Changes in the level of Proteins (mg /g wet wt. of tissue) content in the in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours






Kidney
70
60
50
40
30
20
10
0
mg/g wet .wt of tissue
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III


Muscle
140
120
100
80
60
40
20
0
mg/g. wet . wt. of tissue
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III

3.2.2.Amino acids

3.2.2.1. Gill

Gambusia affinis exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed increase in the amino acid content of the gill when compared with the control group. The percent increase in the content of amino acid in gill with cyfluthrin (group-2) are +9.63 , + 19.61 , + 29 , + 41.93 for 24, 48, 72 and 96 hours respectively. Whereas in *Gambusia affinis* exposed to fenvalerate (group-3) are +12.13, + 23.96 , + 33.21, + 49.31 for 24,48,72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 4: Fig.4).

3.2.2.2. Liver

The content of amino acid in liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a incline , when compare with the control group. The percent incline with cyfluthrin (group-2) are, + 11.34, + 22.43, + 30.08, + 43.98 for 24, 48, 72 and 96 hours respectively. The percent increase with fenvalerate (group-3) are + 12.98, + 24.96, + 34.79, + 52.05 for 24, 48, 72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels) (Table 4: Fig.4).

3.2.2.3. Kidney

In the present study there is a significant increase in the amino acid content in the tissues of kidney , in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of alterations are + 11.7, + 20.38, + 28.05, + 40.46 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .

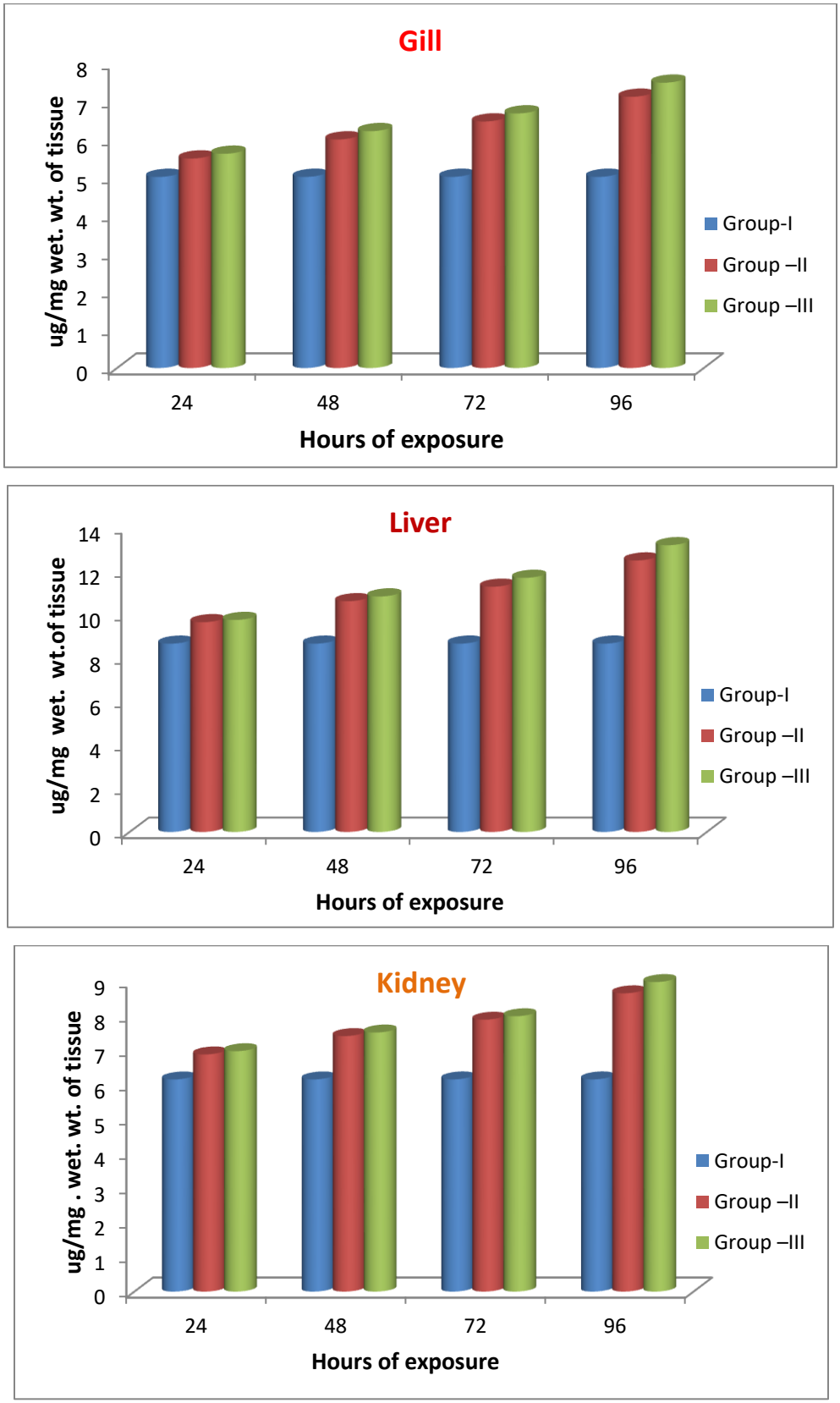
The percentage of alterations in fish exposed to fenvalerate (group-3) are +11.97, + 22.05, + 29.67, + 45.75 for 24, 48, 72 and 96 hours respectively. The levels of protein content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 4: Fig.4).

Table 4 : Variations of amino acid (µg/mg wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control	5.011 ± 0.044	5.012 ± 0.045	5.013 ± 0.047	5.013 ± 0.046
	Group –II- Cyfluthrin % COC	5.494** ± 0.076 % +9.63	5.995** ± 0.078 % + 19.61	6.467** ± 0.079 % + 29	7.115** ± 0.080 % + 41.93
	Group –III- Fenvalerate % COC	5.619** ± 0.065 % +12.13	6.213** ± 0.069 % + 23.96	6.678** ± 0.071 % + 33.21	7.485** ± 0.073 % + 49.31
Liver	Group-I Control	8.670 ± 0.050	8.671 ± 0.051	8.672 ± 0.052	8.672 ± 0.052
	Group –II- Cyfluthrin % COC	9.654** ± 0.043 % + 11.34	10.616**± 0.048 % + 22.43	11.281** ± 0.049 % + 30.08	12.486** ± 0.058 % + 43.98
	Group –III- Fenvalerate % COC	9.757** ± 0.034 % + 12.98	10.836**± 0.038 % + 24.96	11.689** ± 0.039 % + 34.79	13.186** ± 0.041 % + 52.05
Kidney	Group-I Control	6.161 ± 0.036	6.162 ± 0.035	6.163 ± 0.036	6.163 ± 0.037
	Group –II- Cyfluthrin % COC	6.886** ± 0.048 % + 11.7	7.418** ± 0.051 % + 20.38	7.892** ± 0.052 % + 28.05	8.657** ± 0.056 % + 40.46
	Group –III- Fenvalerate % COC	6.980** ± 0.052 % +11.97	7.521** ± 0.054 % + 22.05	7.992** ± 0.056 % + 29.67	8.983** ± 0.056 % + 45.75

Values are mean ± S.E Mean of six individual observations and student t-test. Significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 4 : Changes in the level of amino acid (µg/mg wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours



3.2.3. Tissue Glucose

3.2.3.1.Gill

In the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) the recorded glucose content was increased in the gill when compared with the control group. The percent increased over the control are with cyfluthrin (group-2) are + 7.05, + 18.66, + 25.57, + 38.59 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent increased in glucose over the control are + 9.05, + 20.92, + 27.91, + 41.16 for 24, 48, 72 and 96 hours respectively. The levels of glucose content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 5: Fig.5).

3.2.3.2. Liver

The observed values of glucose content in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a increase , when compare with the control group. The percent incline with cyfluthrin (group-2) are + 11.70, + 20.05 , + 32.33, + 41.80 for 24, 48, 72 and 96 hours respectively. The percent increase with fenvalerate (group-3) are + 12.01, + 21.96 , + 34.37, + 46.82 for 24, 48, 72 and 96 hours respectively. The levels of glucose content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 5: Fig.5).

3.2.3. 3. Kidney

The glucose content of the in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) increased in comparison with control group. The percentage of alterations are + 9.16, + 21.70 , + 29.30, + 37.98 for 24, 48, 72 and 96 hours respectively in fish exposed to

cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are + 12.72, + 23.36, + 31.20, + 42.23 for 24, 48, 72 and 96 hours respectively. The levels of glucose content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 5: Fig.5).

3.2.3..4. Muscle

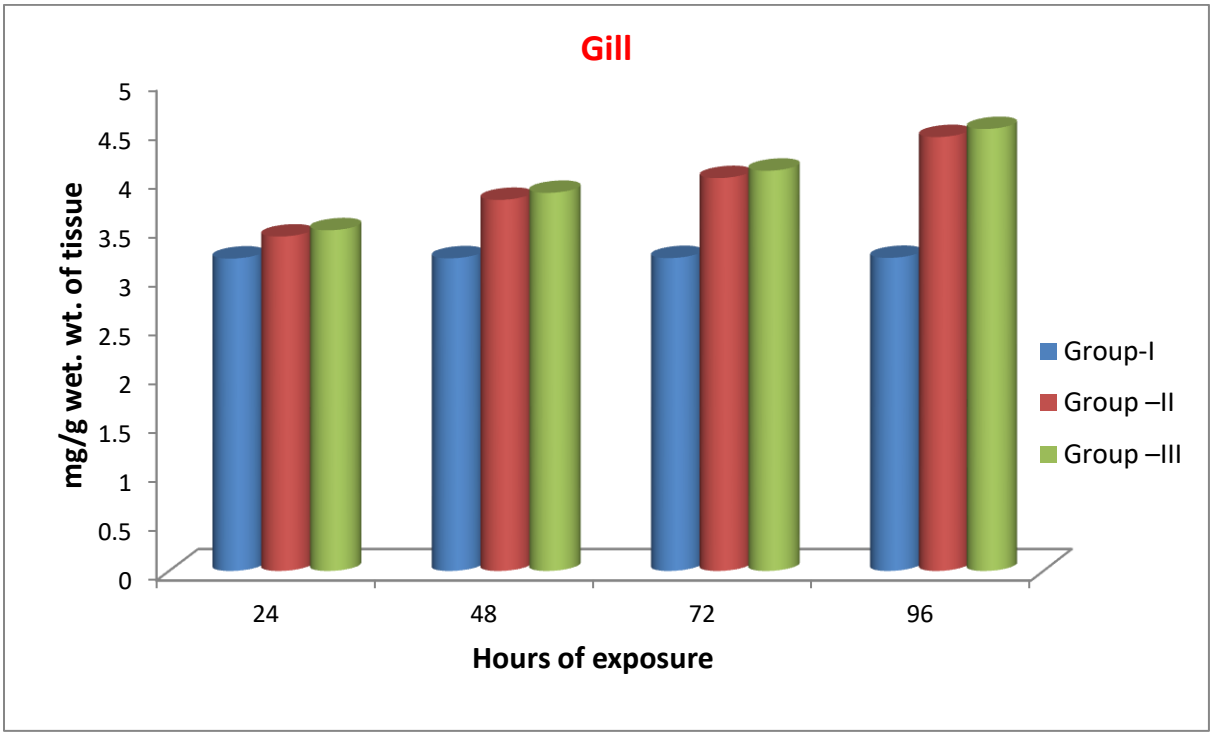
The levels of glucose content in the muscle shown a inclining trend in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of increase in fish exposed to cyfluthrin (group-2) are + 9.28, + 20.37, + 29.58, + 40.48 for 24, 48, 72 and 96 hours respectively. The increase percentage in fish exposed to fenvalerate (group-3) are + 11.92, + 21.78, + 35, + 44.29 for 24, 48, 72 and 96 hours respectively. The levels of glucose content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 5: Fig.5).

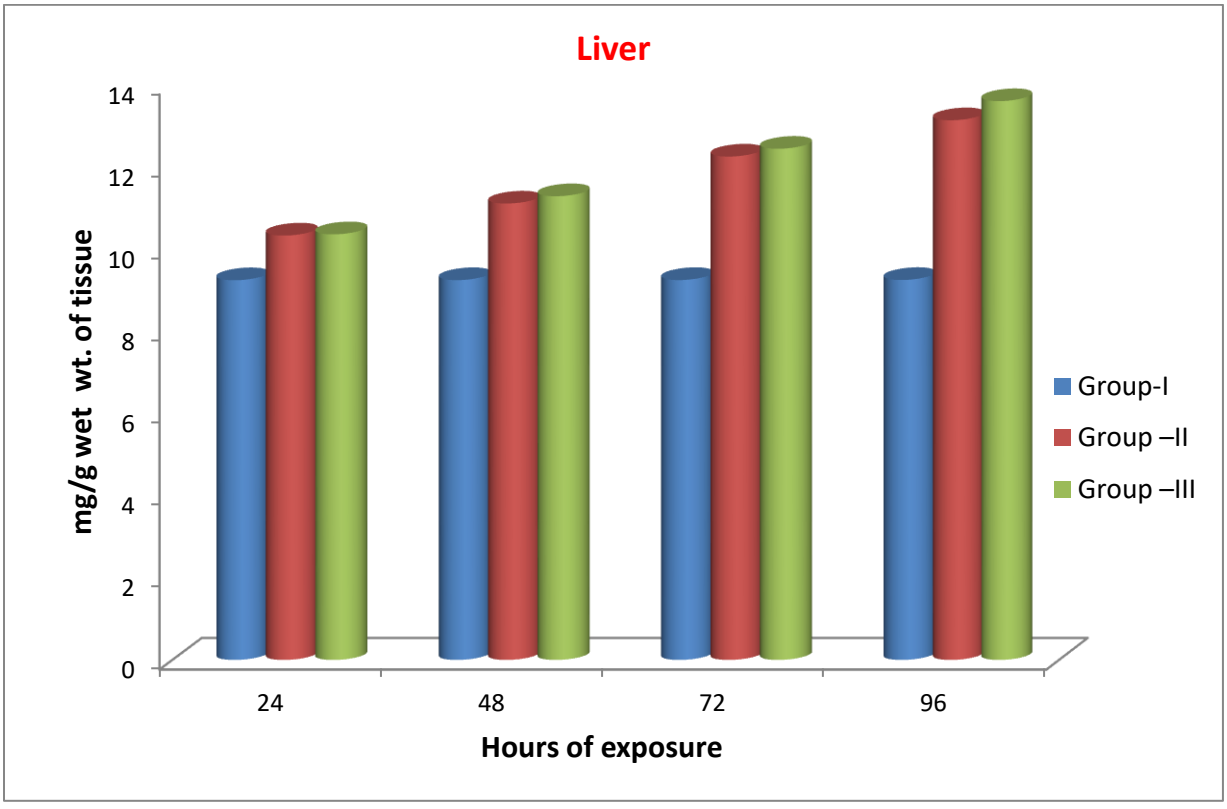
Table 5 : Variations of glucose (mg/g wet. wt. of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

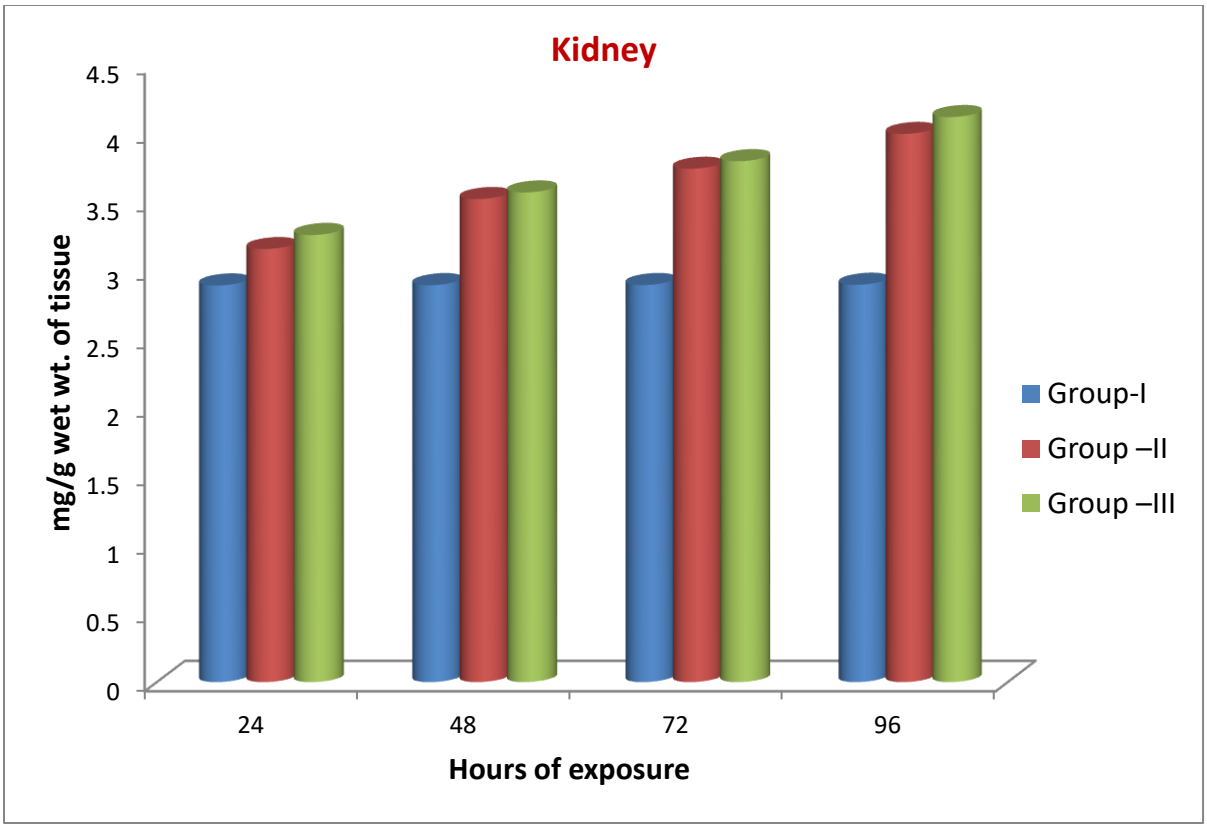
Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control	3.190 ± 0.120	3.193 ± 0.121	3.195 ± 0.123	3.197 ± 0.122
	Group –II- Cyfluthrin % COC	3.415* ± 0.041 % + 7.05	3.789** ± 0.046 % + 18.66	4.012** ± 0.049 % + 25.57	4.431** ± 0.053 % + 38.59
	Group –III- Fenvalerate % COC	3.479* ± 0.062 % + 9.05	3.861** ± 0.065 % + 20.92	4.087** ± 0.067 % + 27.91	4.513** ± 0.069 % + 41.16
Liver	Group-I Control	9.261 ± 0.145	9.263 ± 0.143	9.265 ± 0.143	9.267 ± 0.143
	Group –II- Cyfluthrin % COC	10.345* ± 0.030 % + 11.70	11.121** ± 0.035 % + 20.05	12.261** ± 0.039 % + 32.33	13.141** ± 0.040 % + 41.80
	Group –III- Fenvalerate % COC	10.374* ± 0.071 % + 12.01	11.298** ± 0.074 % + 21.96	12.450** ± 0.076 % + 34.37	13.605** ± 0.081 % + 46.82
Kidney	Group-I Control	2.891 ± 0.182	2.893 ± 0.184	2.894 ± 0.183	2.896 ± 0.183
	Group –II- Cyfluthrin % COC	3.156* ± 0.024 % + 9.16	3.521** ± 0.026 % + 21.70	3.742** ± 0.027 % + 29.30	3.996** ± 0.035 % + 37.98
	Group –III- Fenvalerate % COC	3.259* ± 0.045 % + 12.72	3.569** ± 0.046 % + 23.36	3.797** ± 0.047 % + 31.20	4.119** ± 0.055 % + 42.23
Muscle	Group-I Control	3.674 ± 0.163	3.676 ± 0.164	3.677 ± 0.164	3.678 ± 0.167
	Group –II- Cyfluthrin % COC	4.015* ± 0.076 % + 9.28	4.425** ± 0.081 % + 20.37	4.765** ± 0.091 % + 29.58	5.167** ± 0.081 % + 40.48
	Group –III- Fenvalerate % COC	4.112* ± 0.076 % + 11.92	4.477** ± 0.081 % + 21.78	4.965** ± 0.091 % + 35	5.307** ± 0.081 % + 44.29

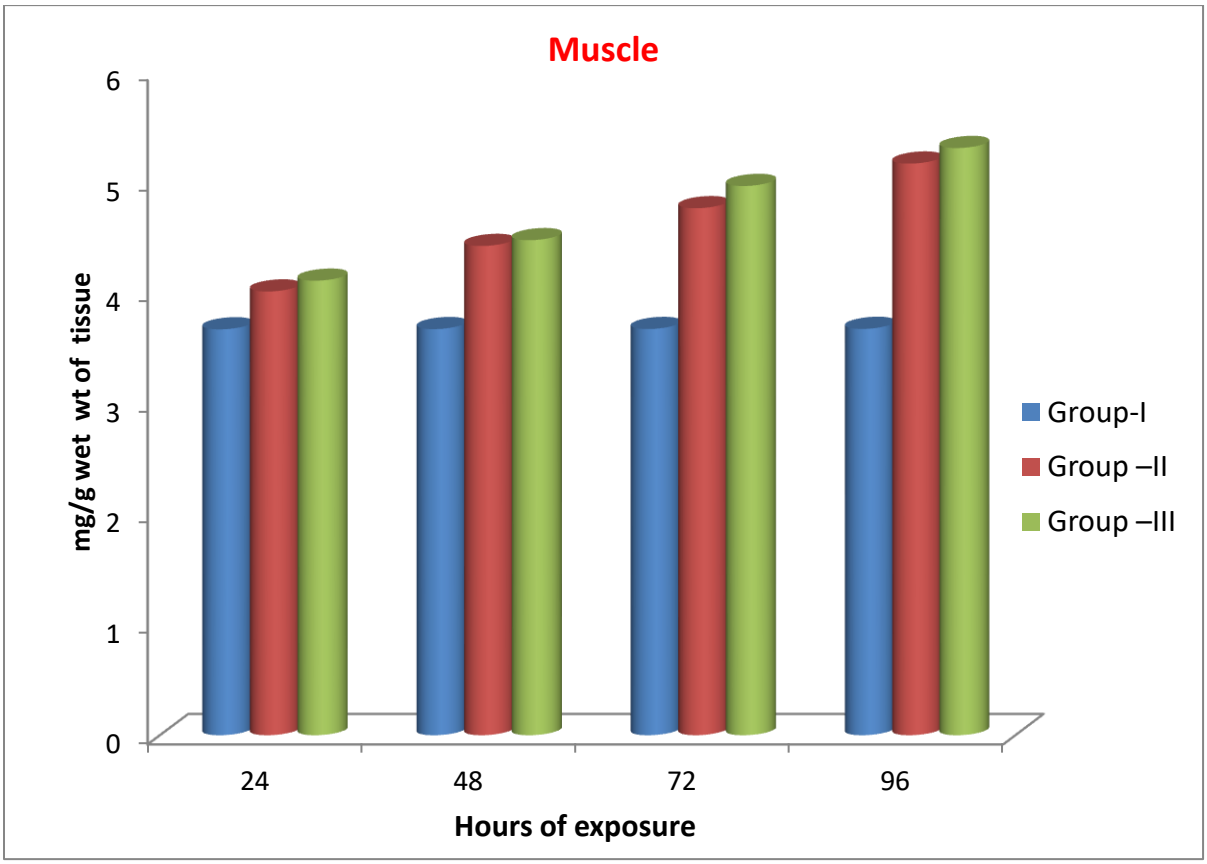
Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased ..%COC (change over control)

Fig. 5 : Changes in the level of glucose (mg/g wet. wt. of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours






Kidney
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
mg/g wet wt. of tissue
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III


Muscle
6
5
4
3
2
1
0
mg/g wet wt of tissue
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III

3.3. ENZYMOLOGICAL PARAMETERS

3.3.1.Succinate dehydrogenase (SDH)

3.3.1.1.Gill

The observed SDH values of gill are represented in Table. 5 and Fig. 5. The observed activity of succinate dehydrogenase (SDH) content in the tissues of gill are decreased in the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control are with cyfluthrin (group-2) are - 7.91, - 13.30, - 17.01, - 21.47 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in SDH activity over the control are - 9.89, - 15.41 , - 16.57, - 24.37 for 24, 48, 72 and 96 hours respectively. The levels of succinate dehydrogenase (SDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 6: Fig.6)..

3.3.1.2. Liver

In the present study succinate dehydrogenase (SDH) acivity in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decrease , when compare with the control group. The percent decline in SDH activity with cyfluthrin (group-2) are - 4.5, - 8.35, - 11.07, - 13.36 for 24, 48, 72 and 96 hours respectively. The percent decrease in SDH activity with fenvalerate (group-3) are - 6.03, - 9.32, - 12.06 , - 15.99 for 24, 48, 72 and 96 hours respectively. The levels of succinate dehydrogenase (SDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 6: Fig.6).

3.3.1.3. Kidney

The succinate dehydrogenase (SDH) content of the in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (

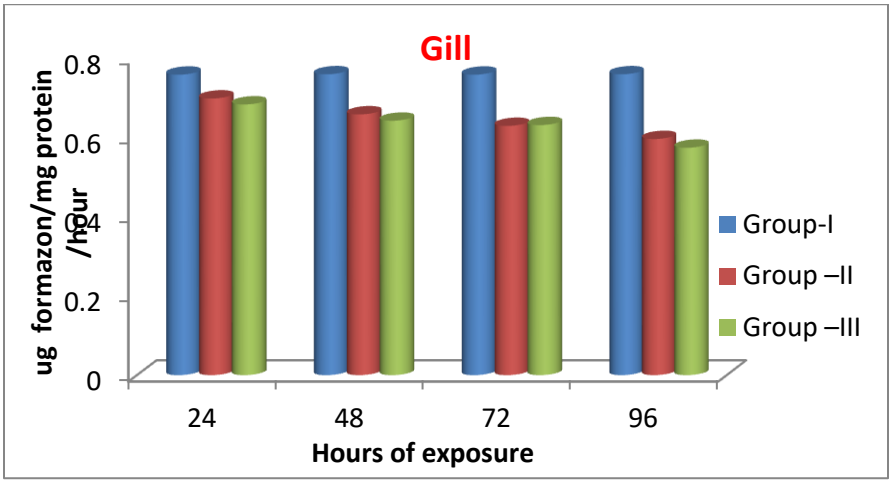
group-3) decreased in comparison with control group. The percentage of alterations are - 2.98, -6.36, - 10.70, - 14.88 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 4.61, - 8.94, - 13.55, - 18.67 for 24, 48, 72 and 96 hours respectively. The levels of succinate dehydrogenase (SDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 6: Fig.6).

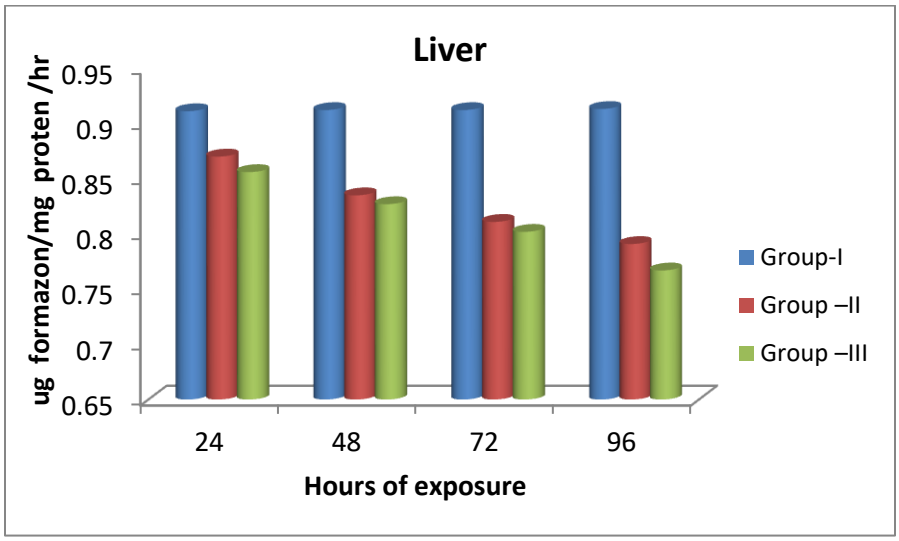
Table 6 : Variations of values Succinate dehydrogenase (SDH) (µg formazon/mg protein/hr) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

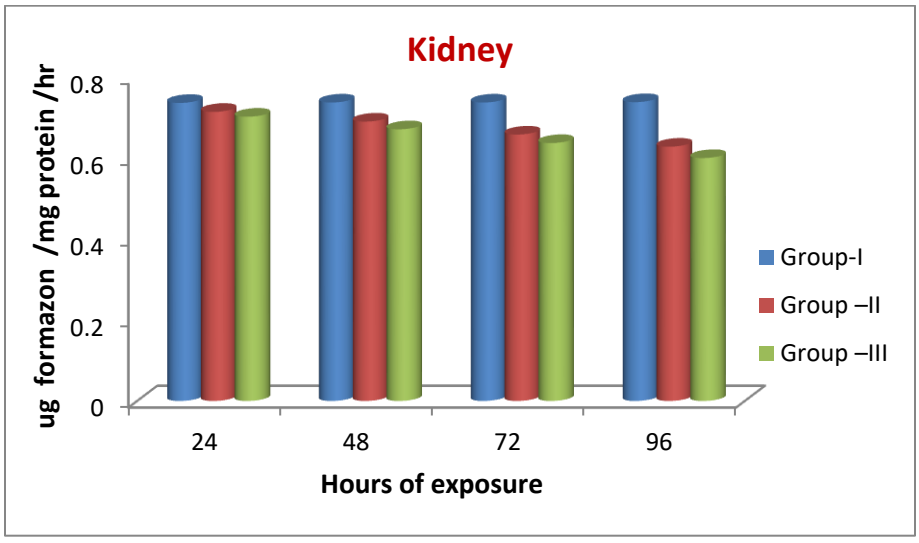
Tissues	Groups	Hours of exposure			
		24	48	72	96
Gill	Group-I Control	0.758 ± 0.002	0.759 ± 0.003	0.758 ± 0.004	0.759 ± 0.003
	Group –II- Cyfluthrin % COC	0.698** ± 0.002 % - 7.91	0.658** ± 0.003 % - 13.30	0.629** ± 0.003 % - 17.01	0.596** ± 0.004 % - 21.47
	Group –III- Fenvalerate % COC	0.683** ± 0.002 % - 9.89	0.642** ± 0.003 % - 15.41	0.631** ± 0.003 % - 16.57	0.574** ± 0.004 % - 24.37
Liver	Group-I Control	0.911 ± 0.001	0.912 ± 0.002	0.912 ± 0.003	0.913 ± 0.003
	Group –II- Cyfluthrin % COC	0.870** ± 0.002 % - 4.5	0.835** ± 0.003 % - 8.35	0.811** ± 0.002 % - 11.07	0.791** ± 0.003 % - 13.36
	Group –III- Fenvalerate % COC	0.856** ± 0.001 % - 6.03	0.827** ± 0.002 % - 9.32	0.802** ± 0.002 % - 12.06	0.767** ± 0.003 % - 15.99
Kidney	Group-I Control	0.737 ± 0.002	0.738 ± 0.003	0.738 ± 0.002	0.739 ± 0.003
	Group –II- Cyfluthrin % COC	0.715** ± 0.002 % - 2.98	0.691** ± 0.003 % -6.36	0.659** ± 0.002 % - 10.70	0.629** ± 0.002 % - 14.88
	Group –III- Fenvalerate % COC	0.703** ± 0.003 % - 4.61	0.672** ± 0.002 % - 8.94	0.638** ± 0.003 % - 13.55	0.601** ± 0.003 % - 18.67

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased ..%COC (change over control)

Fig. 6 : Changes in the level of Succinate dehydrogenase (SDH) (µg formazon/mg protein/hr) activity in *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours







3.3.2. Lactate dehydrogenase (LDH)

3.3.2.1. Gill

The observation of LDH activity in the tissues of gill are decreased in the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control with cyfluthrin (group-2) are - 6.77, -17.61, - 25.25, - 29.38 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in lacatate dehydrogenase (LDH) over the control are - 7.8, - 18.65, - 24.75, - 30.92 for 24, 48, 72 and 96 hours respectively. The levels of lactate dehydrogenase (LDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 7: Fig.7)..

3.3.2.1. Liver

The observed value of lactate dehydrogenase (LDH) acivity in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percent decline in LDH activity with cyfluthrin (group-2) are - 3.2, - 6.32, -9.30, - 12.95 for 24, 48, 72 and 96 hours respectively. The percent decrease in LDH activity with fenvalerate (group-3) are - 4.22, - 8.73, - 11.41, - 16.26 for 24, 48, 72 and 96 hours respectively. The levels of lactate dehydrogenase (LDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 7: Fig.7).

3.3.2.1.Kidney

The lactate dehydrogenase (LDH)) content of the in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 5.96, - 9.86, - 15.03, - 18.30 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .

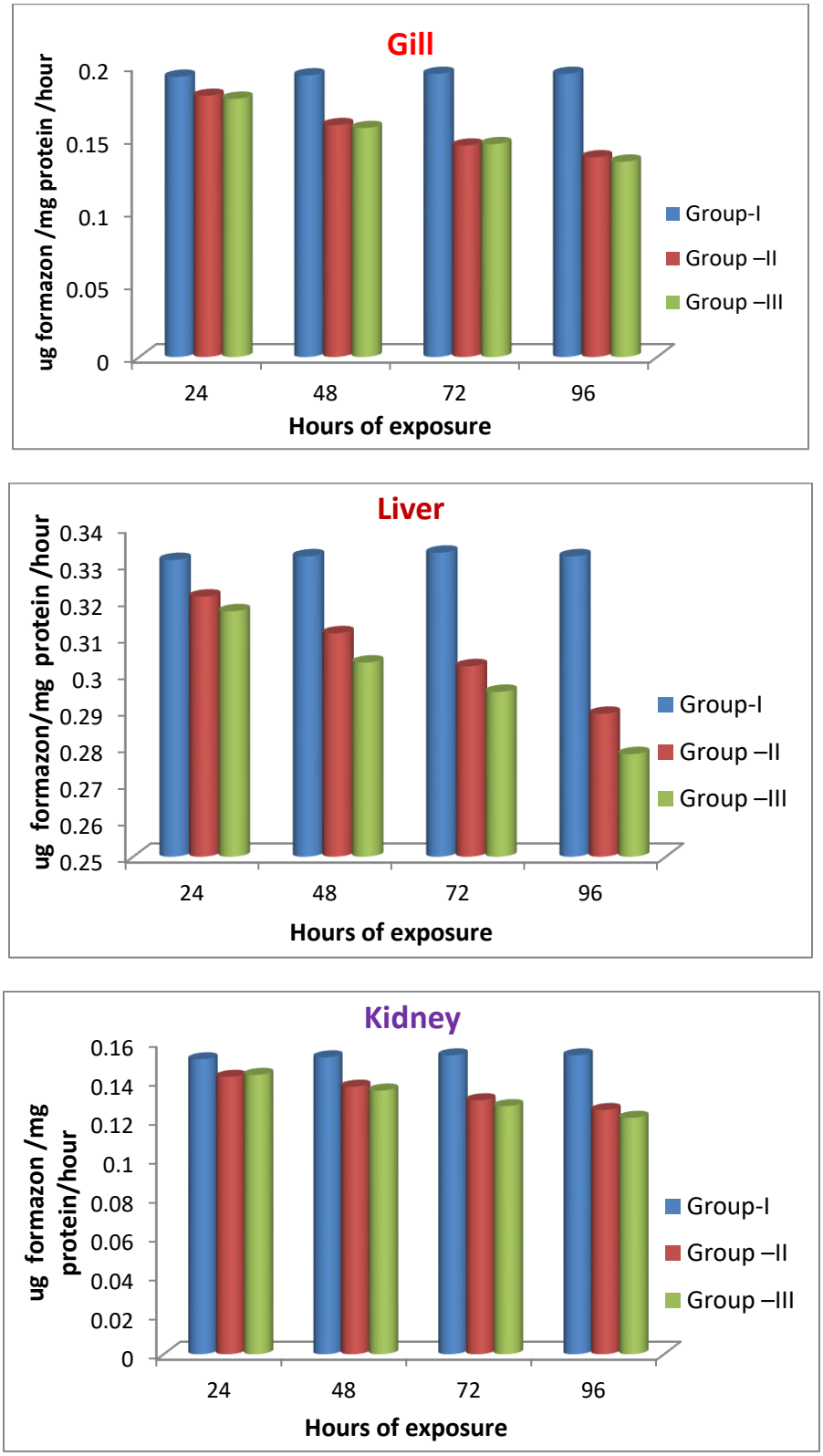
The percentage of alterations in fish exposed to fenvalerate (group-3) are - 5.29, - 11.18, - 17, - 20.91 for 24, 48, 72 and 96 hours respectively. The levels of lactate dehydrogenase (LDH) content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 7: Fig.7).

Table 7 : Variations of values Lactate dehydrogenase (LDH) (µg formazon/mg protein/hour) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control	0.192 ± 0.002	0.193 ± 0.003	0.194 ± 0.003	0.194 ± 0.002
	Group –II- Cyfluthrin % COC	0.179** ± 0.003 % - 6.77	0.159**± 0.004 % -17.61	0.145** ± 0.004 % - 25.25	0.137** ± 0.003 % - 29.38
	Group –III- Fenvalerate % COC	0.177** ± 0.002 % - 7.8	0.157**± 0.003 % - 18.65	0.146** ± 0.003 % - 24.75	0.134** ± 0.004 % - 30.92
Liver	Group-I Control	0.331 ± 0.004	0.332 ± 0.003	0.333 ± 0.002	0.332 ± 0.003
	Group –II- Cyfluthrin % COC	0.321** ± 0.003 % - 3.2	0.311**± 0.002 % - 6.32	0.302** ± 0.002 % -9.30	0.289** ± 0.004 % - 12.95
	Group –III- Fenvalerate % COC	0.317** ± 0.002 % - 4.22	0.303**± 0.003 % - 8.73	0.295** ± 0.003 % - 11.41	0.278** ± 0.002 % - 16.26
Kidney	Group-I Control	0.151 ± 0.003	0.152 ± 0.002	0.153 ± 0.004	0.153 ± 0.003
	Group –II- Cyfluthrin % COC	0.142** ± 0.001 % - 5.96	0.137** ± 0.002 % - 9.86	0.130** ± 0.002 % - 15.03	0.125** ± 0.002 % - 18.30
	Group –III- Fenvalerate % COC	0.143** ± 0.002 % - 5.29	0.135**± 0.003 % - 11.18	0.127** ± 0.002 % - 17	0.121** ± 0.002 % - 20.91

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased, %COC (change over control) ;

Fig. 7 : Changes in the level of Lactate dehydrogenase (LDH) (µg formazon/mg protein/hr) activity in *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours



3.3.3. Glucose-6 phosphatase

3.3.3.1.Liver

Glucose 6 phosphatase content of the in the tissues of liver in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of decrease are - 11.87, - 16.7, - 22.16, - 26.17 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 12.89 , - 17.15, - 21.90, - 27.87 for 24, 48, 72 and 96 hours respectively. The levels of Glucose 6 phosphatase content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 8: Fig.8).

3.3.3.2.Kidney

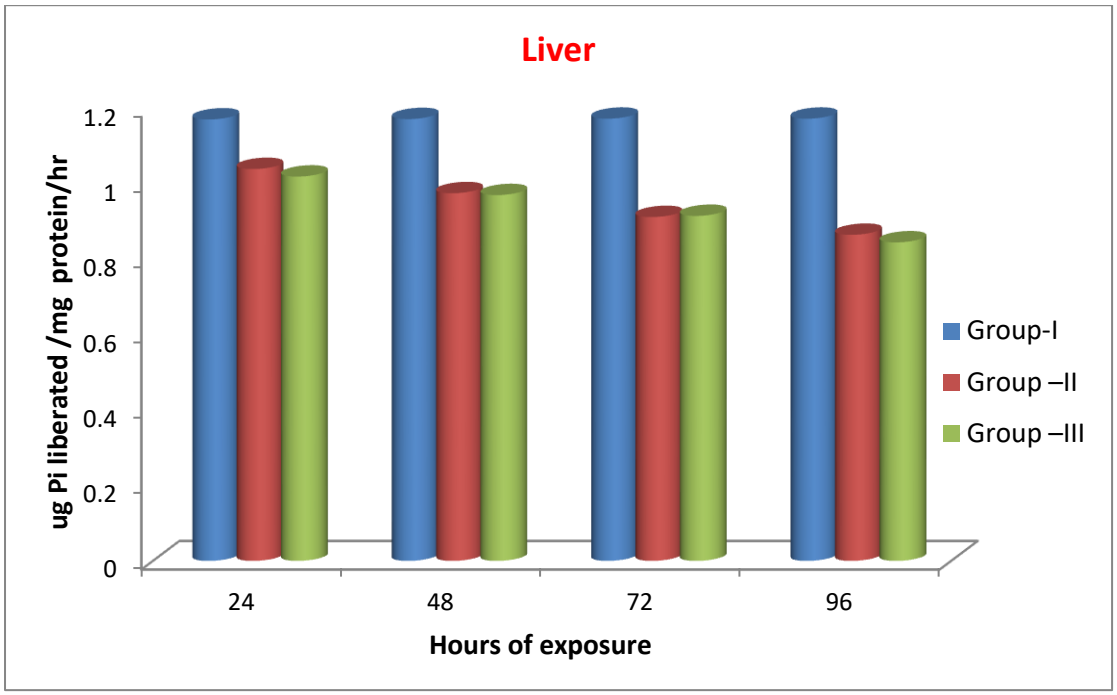
The observed value of Glucose 6 phosphatase acivity in the kidney tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percentage of decline in Glucose 6 phosphatase activity with cyfluthrin (group-2) are - 8.02, - 15.53, - 21.33, - 25.86 for 24, 48, 72 and 96 hours respectively. The percent decrease in Glucose 6 phosphatase activity with fenvalerate (group-3) are - 10.64, - 16.64, - 21.18 , - 27.53 for 24, 48, 72 and 96 hours respectively. The levels of Glucose 6 phosphatase content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 8: Fig.8).

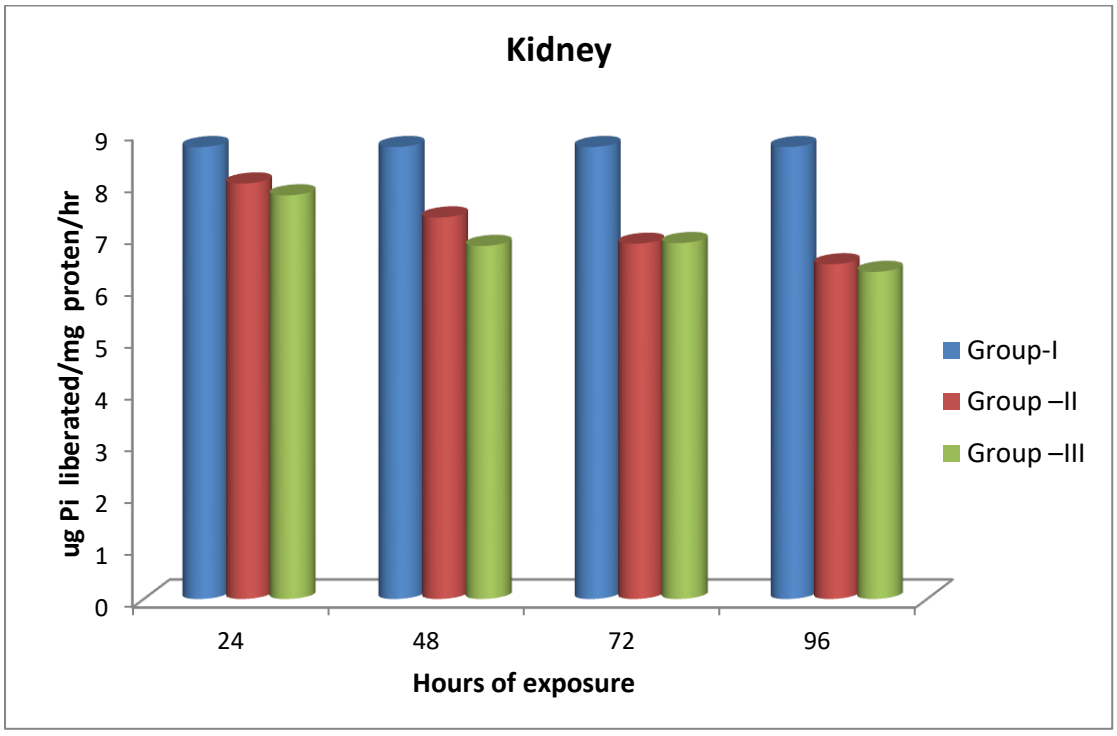
Table 8 : Variations of glucose-6 phosphatase (μg Pi liberated/mg protein/h.) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Liver	Group-I Control	1.171 ± 0.023	1.172 ± 0.024	1.173 ± 0.024	1.173 ± 0.025
	Group –II- Cyfluthrin % COC	1.040* ± 0.030 % - 11.87	0.976** ± 0.055 % - 16.7	0.913** ± 0.022 % - 22.16	0.866** ± 0.060 % - 26.17
	Group –III- Fenvalerate % COC	1.02* ± 0.014 % - 12.89	0.971* ± 0.021 % - 17.15	0.916* ± 0.034 % - 21.90	*0.846 ± 0.030 % - 27.87
Kidney	Group-I Control	8.711 ± 0.052	8.712 ± 0.053	8.713 ± 0.053	8.712 ± 0.054
	Group –II- Cyfluthrin % COC	8.012** ± 0.024 % - 8.02	7.359** ± 0.026 % - 15.53	6.854** ± 0.025 % - 21.33	6.459** ± 0.025 % - 25.86
	Group –III- Fenvalerate % COC	7.784** ± 0.031 % - 10.64	6.812** ± 0.032 % - 16.64	7.015** ± 0.33 % - 19.48	6.713** ± 0.036 % - 22.94

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased ..%COC (change over control)

Fig. 8 : Changes in the level **of glucose-6 phosphatase (µg Pi liberated/mg protein/h.) activity in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours**





3.3.4.Acetylcholinesterase (AChE)

3.3.4.1.Gill

In the present observation when *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed significant decrease in the levels of AChE activity content in the gill when compared with the control group. The percent changes over the control with cyfluthrin (group-2) are - 10.21 , - 18.97, - 28.62, - 36.08 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decrease in AChE activity over the control are - 12.41, - 20.67, - 31.43, - 40.96 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 9: Fig.9).

3.3.4.2. Liver

In the present study the observed values of AChE activity in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a significant decline ,when compare with the control group. The percent decline with cyfluthrin (group-2) are -8.89 , - 16.23, - 26.72, - 34.67 for 24, 48, 72 and 96 hours respectively. The percent decrease with fenvalerate (group-3) are - 9.71, - 17.67, - 29.60,- 34.11 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 9: Fig.9).

3.3.4.3.Kidney

The AChE activity in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 12.65, - 21.37, - 28.99, - 43.33 for 24, 48, 72 and 96 hours respectively in fish exposed to

cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 13.88, - 23.85, - 31.44, - 37 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 9: Fig.9).

3.3.4.4. Muscle

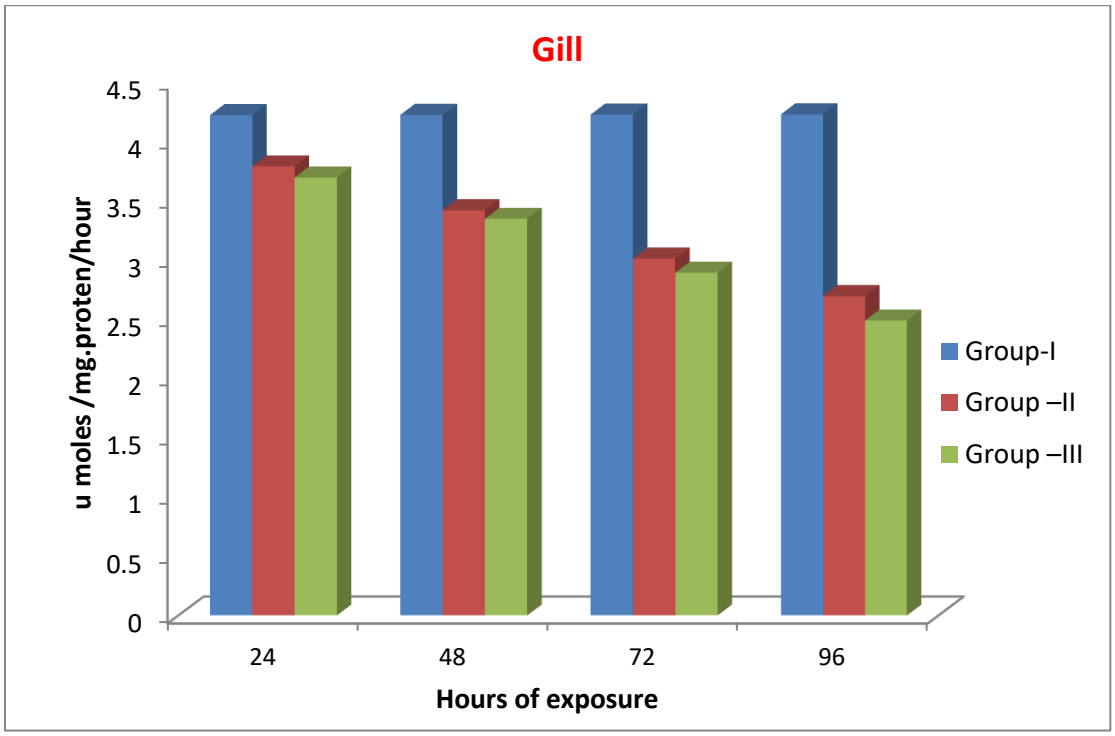
The levels of AChE activity in the muscle shown a declining trend in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of decline in fish exposed to cyfluthrin (group-2) are - 8.46, - 15.86, - 24.59, - 33.11 for 24, 48, 72 and 96 hours respectively. The decline percentage in fish exposed to fenvalerate (group-3) are - 9.60, - 16.56, - 23.41, - 34.62 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 9: Fig.9).

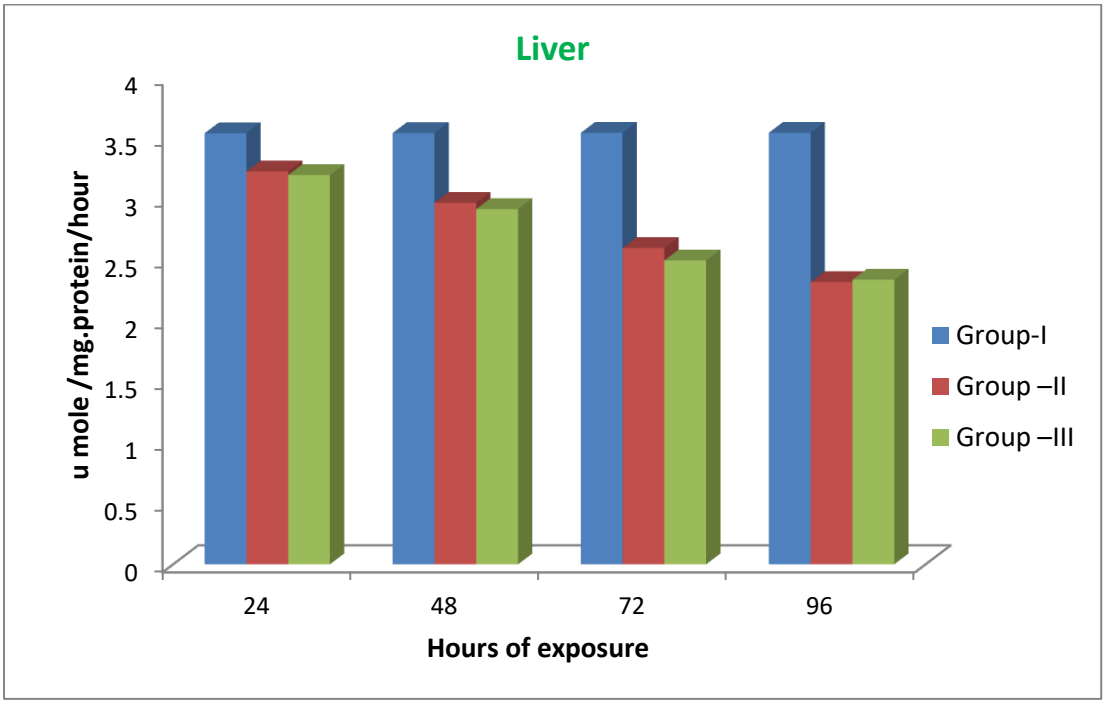
Table 9 : Variations of Acetylcholinesterase (AChE) (umole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

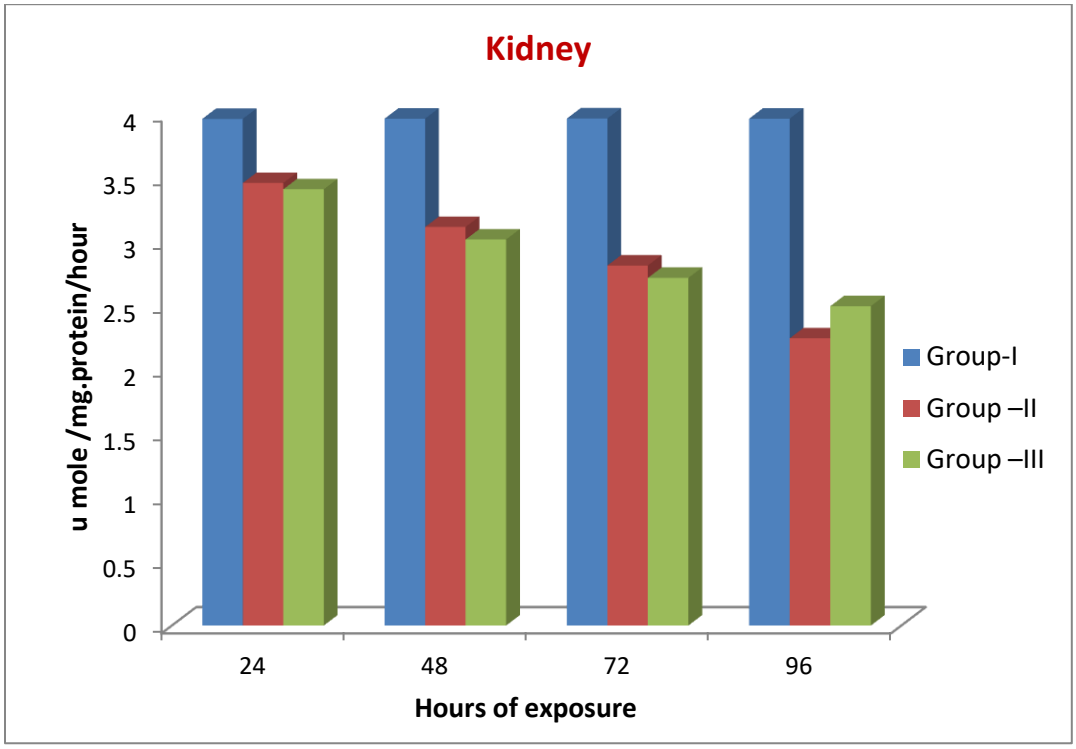
Tissues	Groups	Hours of exposure			
		24	48	72	96
Gill	Group-I Control	4.220 ± 0.056	4.222 ± 0.057	4.224 ± 0.058	4.226 ± 0.059
	Group –II- Cyfluthrin % COC	3.789** ± 0.034 % - 10.21	3.421** ± 0.033 % - 18.97	3.015** ± 0.036 % - 28.62	2.701** ± 0.034 % - 36.08
	Group –III- Fenvalerate % COC	3.696** ± 0.044 % - 12.41	3.349** ± 0.046 % - 20.67	2.896** ± 0.046 % - 31.43	2.495** ± 0.047 % - 40.96
Liver	Group-I Control	3.540 ± 0.071	3.542 ± 0.073	3.543 ± 0.076	3.544 ± 0.079
	Group –II- Cyfluthrin % COC	3.225** ± 0.048 % -8.89	2.967** ± 0.051 % - 16.23	2.596** ± 0.053 % - 26.72	2.315** ± 0.061 % - 34.67
	Group –III- Fenvalerate % COC	3.196** ± 0.027 % - 9.71	2.916** ± 0.028 % - 17.67	2.494** ± 0.031 % - 29.60	2.335** ± 0.034 % - 34.11
Kidney	Group-I Control	3.960 ± 0.029	3.962 ± 0.029	3.963 ± 0.034	3.962 ± 0.037
	Group –II- Cyfluthrin % COC	3.459** ± 0.066 % - 12.65	3.115** ± 0.068 % - 21.37	2.814** ± 0.072 % - 28.99	2.245** ± 0.076 % - 43.33
	Group –III- Fenvalerate % COC	3.410** ± 0.091 % - 13.88	3.017** ± 0.092 % - 23.85	2.717** ± 0.092 % - 31.44	2.496** ± 0.096 % - 37
Brain	Group-I Control	4.581 ± 0.081	4.583 ± 0.082	4.583 ± 0.085	4.584 ± 0.086
	Group –II- Cyfluthrin % COC	4.193** ± 0.062 % - 8.46	3.856** ± 0.065 % - 15.86	3.456** ± 0.072 % - 24.59	3.066** ± 0.076 % - 33.11
	Group –III- Fenvalerate % COC	4.141** ± 0.074 % - 9.60	3.824** ± 0.077 % - 16.56	3.510** ± 0.079 % - 23.41	2.997** ± 0.080 % - 34.62

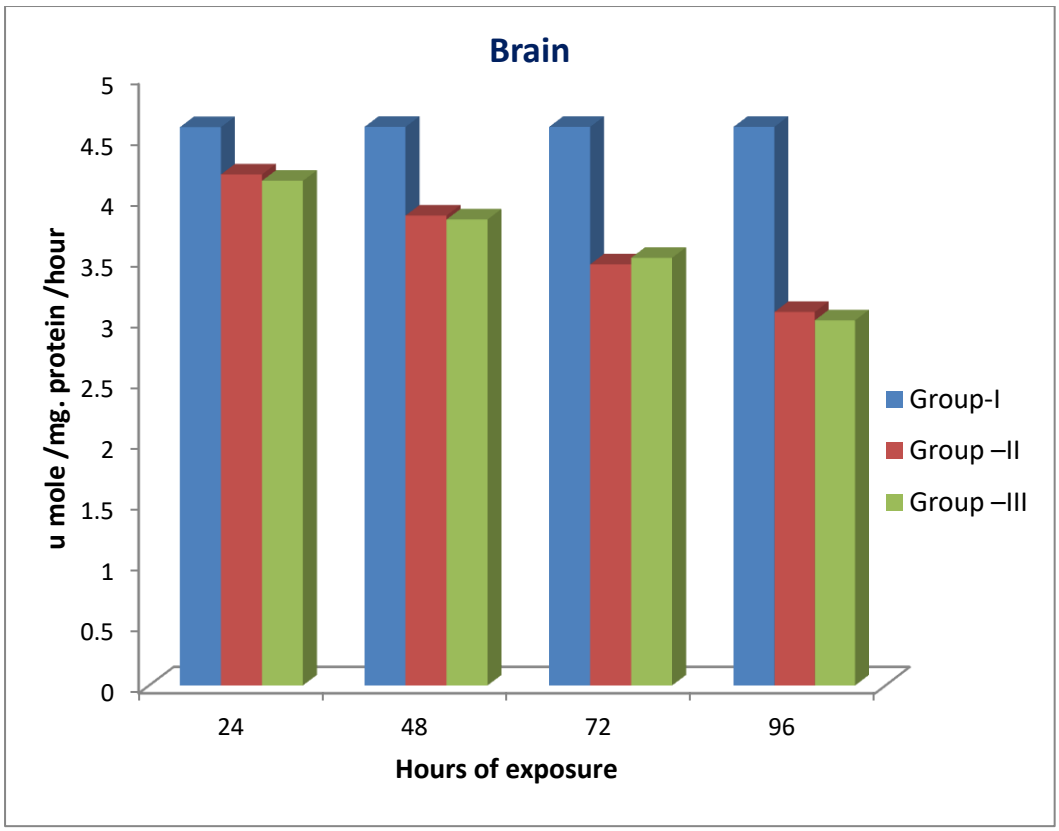
Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 9 : Changes in the level of Acetylcholinesterase (AChE) (u mole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours






Kidney
u mole /mg.protein/hour
0
0.5
1
1.5
2
2.5
3
3.5
4
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III


Brain
u mole /mg. protein /hour
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
24
48
72
96
Hours of exposure
Group-I
Group –II
Group –III

3.4.NUCLEIC ACIDS

3.4.1. Deoxyribonucleic acid (DNA)

3.4.1.1.Gill

The content of DNA in gill tissues are decreased significantly in the present study when *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control with cyfluthrin (group-2) are - 16.73, - 22.22, - 27.08, - 32.35 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in DNA content over the control are -20.21, 30.05, - 39.19, - 43.94 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 10: Fig.10).

3.4.1.2. Liver

The observed value of DNA content in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percent decline in DNA content with cyfluthrin (group-2) are- 19.48, - 27.88, - 30.37, - 38.63 for 24, 48, 72 and 96 hours respectively. The percent decrease in DNA content with fenvalerate (group-3) are - 23.84, - 32.38, - 45.33 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels ((Table 10: Fig.10).

3.4.1.3. Kidney

The DNA content of the in the tissues of kidney in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are -9.92, - 16.83, - 19.62, - 23.90 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .

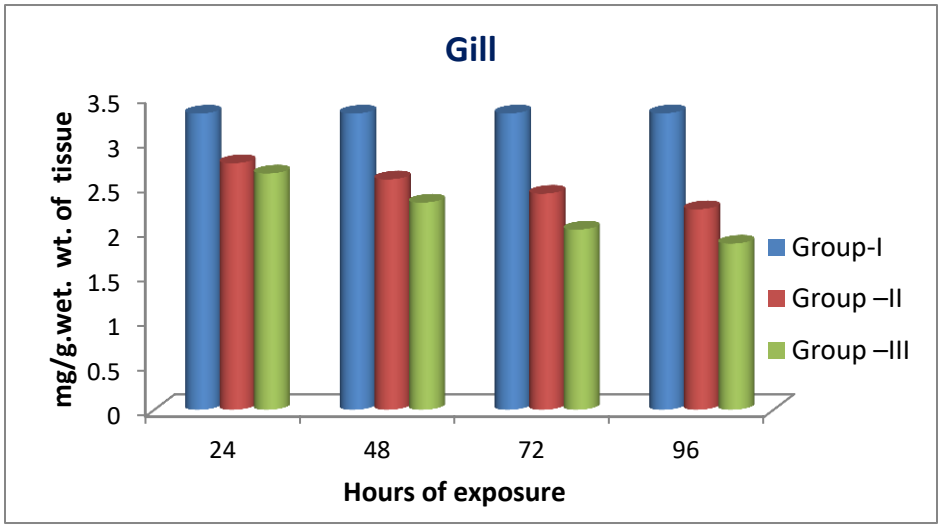
The percentage of alterations in fish exposed to fenvalerate (group-3) are - 11.26, - 19.57, - 28.07, - 34.39 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 10: Fig.10).

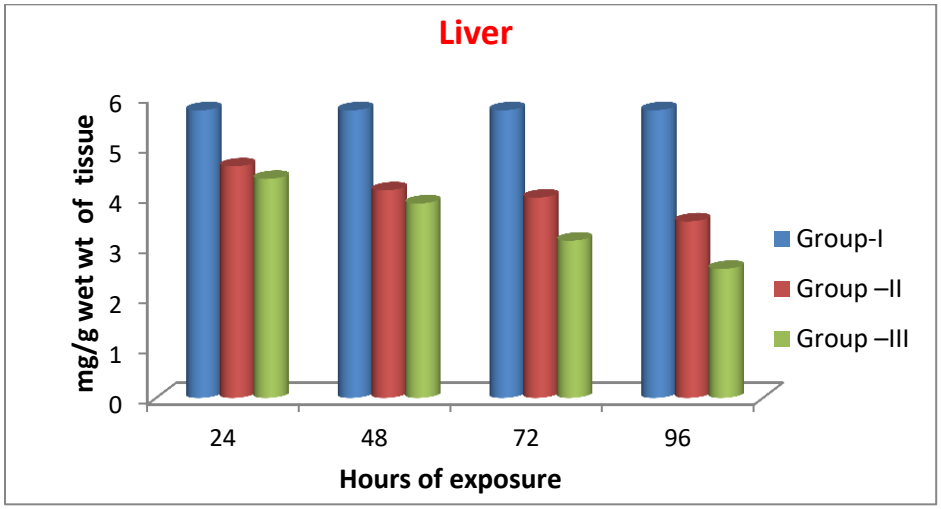
Table 10 : Variations of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) content in fresh water fish *Gambusia affinis* *exposed to* Cyfluthrin and Fenvalerate for 96 hours

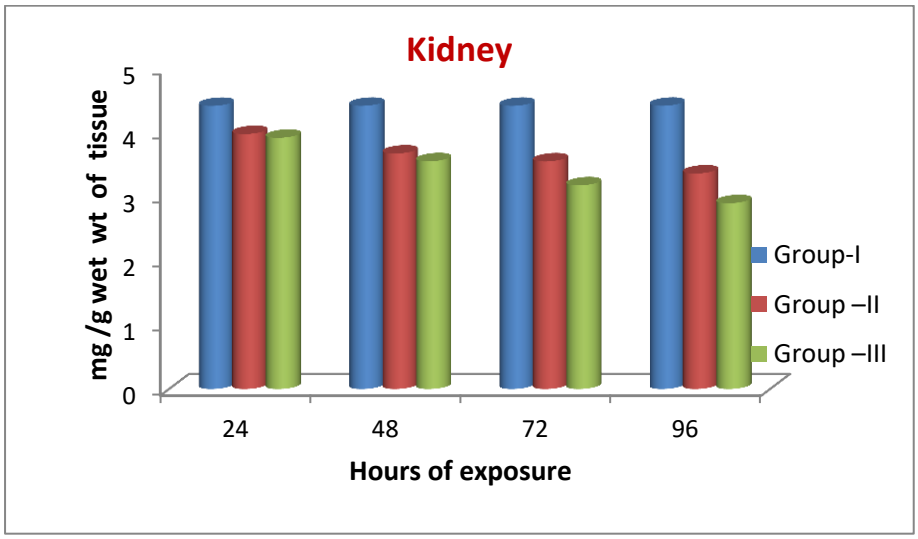
Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control	3.310 ± 0.045	3.311 ± 0.043	3.312 ± 0.042	3.313 ± 0.040
	Group –II- Cyfluthrin % COC	2.756* ± 0.065 % - 16.73	2.575** ± 0.064 % - 22.22	2.415** ± 0.065 % - 27.08	2.241** ± 0.066 % - 32.35
	Group –III- Fenvalerate % COC	2.641* ± 0.065 % -20.21	2.316** ± 0.064 % -30.05	2.014** ± 0.065 % - 39.19	1.857** ± 0.066 % - 43.94
Liver	Group-I Control	5.721± 0.674	5.722± 0.672	5.722± 0.671	5.723± 0.669
	Group –II- Cyfluthrin % COC	4.606* ± 0.733 % - 19.48	4.129** ± 0.737 % - 27.88	3.984** ± 0.739 % - 30.37	3.512** ± 0.740 % - 38.63
	Group –III- Fenvalerate % COC	4.357* ± 0.733 % - 23.84	3.869** ± 0.737 % - 32.38	3.128** ± 0.739 % - 45.33	2.567** ± 0.740 % -55.14
Kidney	Group-I Control	4.412 ± 0.049	4.413 ± 0.047	4.413 ± 0.046	4.414 ± 0.048
	Group –II- Cyfluthrin % COC	3.974* ± 0.670 % -9.92	3.670* ± 0.672 % - 16.83	3.547** ± 0.674 % - 19.62	3.359** ± 0.680 % - 23.90
	Group –III- Fenvalerate % COC	3.915* ± 0.542 % - 11.26	3.549* ± 0.546 % - 19.57	3.174* ± 0.550 % - 28.07	2.896* ± 0.553 % - 34.39

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 10 : Changes in the content of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours







3.4.2. Ribonucleic acid (RNA)

3.4.2.1. Gill

In the present study RNA content in the tissues of gill are decreased in the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control with cyfluthrin (group-2) are - 4.93, - 6.17, -10.65, - 17.066, for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in RNA content over the control are - 6.17, - 9.05, - 14.34, - 19.6 for 24, 48, 72 and 96 hours respectively. The levels of RNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 11: Fig.11).

3.4.2.2.Liver

The observed value of RNA content in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percent decline in RNA content with cyfluthrin (group-2) are - 3.41, - 6.41, - 9.83, - 12.24 for 24, 48, 72 and 96 hours respectively. The percent decrease in RNA content with fenvalerate (group-3) are - 4.61, - 9.03, - 12.24, - 15.63 for 24, 48, 72 and 96 hours respectively. The levels of RNA content content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 11: Fig.11).

3.4.2.3. Kidney

In the present investigation the RNA content of the in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 2.90, - 5.78, - 8.68, - 12.17 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2).

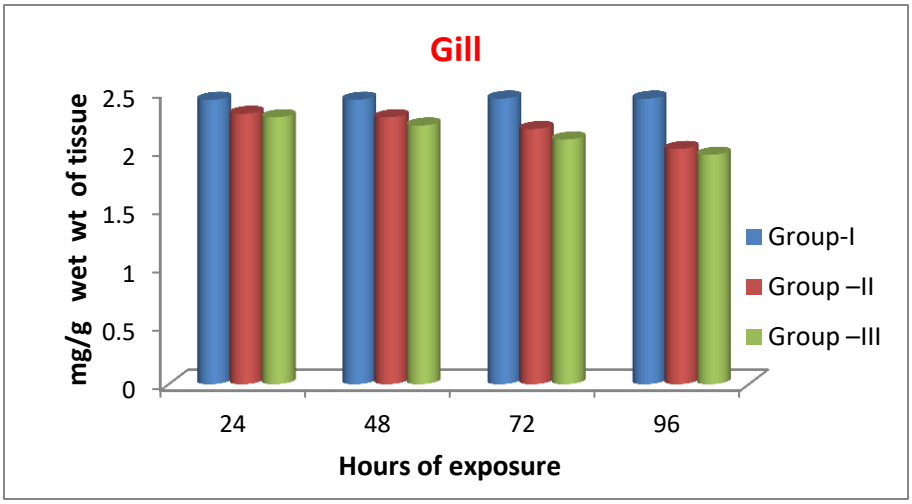
The percentage of alterations in fish exposed to fenvalerate (group-3) are - 4.18, - 6.75, - 9.64, - 13.78 for 24, 48, 72 and 96 hours respectively. The levels of RNA content content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 11: Fig.11).

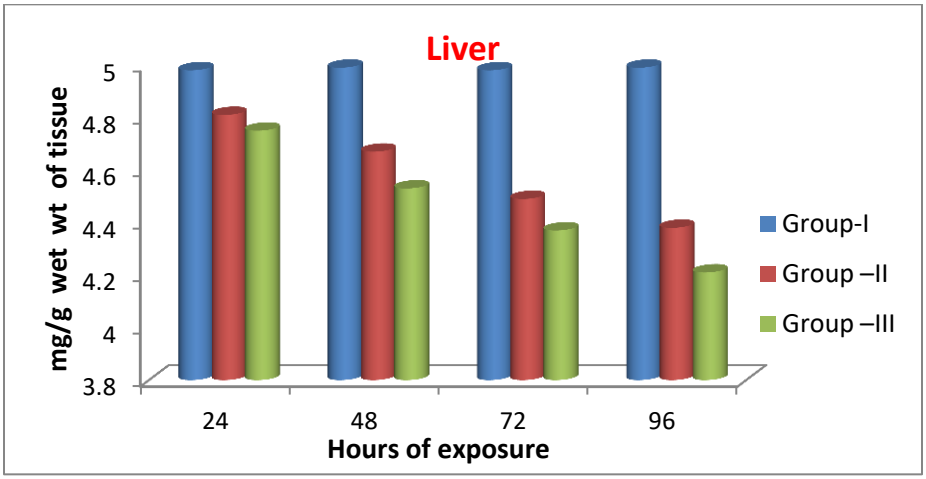
Table 11 : Variations of Ribonucleic acid (RNA) (mg/g wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

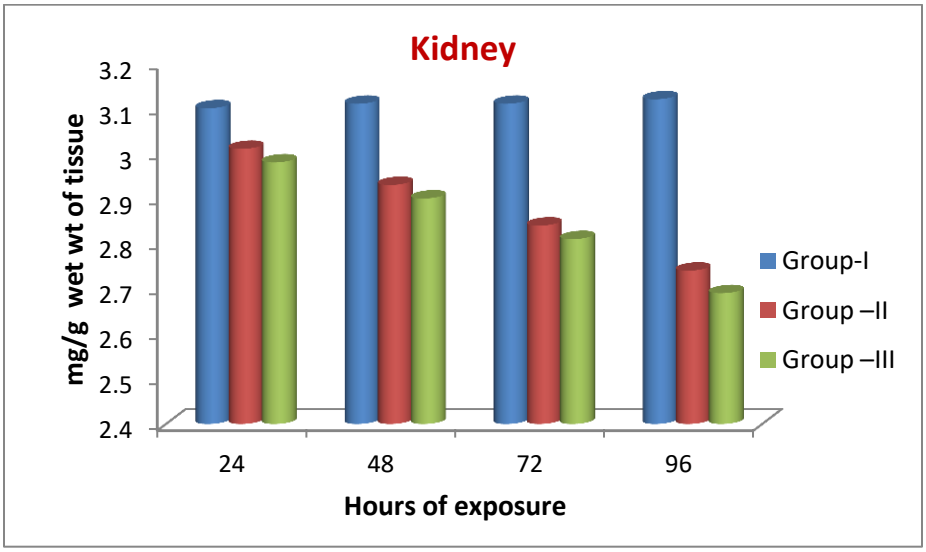
Tissues	Groups	Hours of exposure			
		24	**48**	**72**	**96**
Gill	Group-I Control	2.43± 0.121	2.43± 0.122	2.44± 0.122	2.44± 0.123
	Group –II- Cyfluthrin % COC	2.31* ± 0.145 % - 4.93	2.28* ± 0.146 % - 6.17	2.18** ± 0.145 % - 10.65	2.01** ± 0.145 % - 17.066
	Group –III- Fenvalerate % COC	2.28* ± 0.453 % - 6.17	2.21* ± 0.456 % - 9.05	2.09** ± 0.458 % - 14.34	1.96** ± 0.457 % - 19.6
Liver	Group-I Control	4.98 ± 0.221	4.99 ± 0.222	4.98 ± 0.224	4.99 ± 0.223
	Group –II- Cyfluthrin % COC	4.81* ± 0.323 % - 3.41	4.67* ± 0.324 % - 6.41	4.49** ± 0.324 % - 9.83	4.38** ± 0.326 % - 12.24
	Group –III- Fenvalerate % COC	4.75* ± 0.452 % - 4.61	4.53* ± 0.457 % - 9.03	4.37** ± 0.458 % - 12.24	4.21** ± 0.459 % - 15.63
Kidney	Group-I Control	3.10 ± 0.115	3.11 ± 0.114	3.11 ± 0.115	3.12 ± 0.116
	Group –II- Cyfluthrin % COC	3.01*± 0.145 % - 2.90	2.93* ± 0.147 % - 5.78	2.84 **± 0.148 % - 8.68	2.74** ± 0.149 % - 12.17
	Group –III- Fenvalerate % COC	2.98*± 0.261 % - 4.18	2.90* ± 0.263 % - 6.75	2.81 **± 0.267 % - 9.64	2.69** ± 0.298 % - 13.78

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels(+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 11 : Changes in the content of Ribonucleic acid (RNA) (mg/g wet. wt of tissue) in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours







CHAPTER - 3

DISCUSSION

Pesticides deliberately added to the environment for eradication of pests but they are entering in to the environment affecting the non target organism like fishes . So fishes are used widely for the bioassay of these pesticides .

Pollutants, changes in the environmental toxicity of surrounding water bring pathological changes in the blood of fishes. Hematological parameters are important for toxicological research and as indicators of environmental stress and disease in fish (Kumar , 1999a, b, 2004; Talas and Gulhan, 2009). Pesticide pollution affects the blood and resulted in decline of RBC (Talas and Gulhan, 2009; Joshi , 1988; Joshi , 2002; Johal and Grewal, 2004; Gautam and Kumar, 2008).

Blood parameters of fish are altered by the pescticides. In the present study a significant decrease in RBC,Hb and PCV contents of the blood were observed when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate .The erythropoetic tissue of the fish is disrupted by these pesticides which results in the decline of erythrocyte count. A similar observation was reported by Banaee 2012.

Studies reveals that RBC count Hb concentration in the blood depends on several factors like age of the anima;, stress , sec, availability of food (Saxena and Seth., 2002). The decrease in the haematological parmeters Hb,RBC and PCV may be due to the haemolysis and shrinkage of RBC by OP resulting in anemia . A similar observation was reported in *Clarias gariepinus* treated with endosulfan (Yekeen TA and Fawole, 2011). Similar reduction in RBC was reported when *Labeo rohita* exposed to cypermethrin. (Das & Mukherjee 2005). In the present study PCV values decreased when exposed to pesticides ,due to major reduction in RBC . A Similar reduction in PCV was reported when Tilapia guineensis exposed to cypermethrin (Chindah , 2004).

A Similar observation was made in the haemoglobin contents and packed cell volume of fish species such as *Cyprinus carpio, Puntius ticto, Tor putitora, Oreochromis mossambicus, Mystus keletiusto, Channa punctatus Ophiocephalus punctatus, Labeo rohita* and *Heteropneustes fossilis* (Bansal, 1979; Abidi and Srivastava, 1980; Agrawal and Tyagi, 1988; Bhatkar and Dhande, 2000; Anandkumar, 2001; Bhatia, 2002, 2004; Sampath, 2003; Satyanarayan, 2004; Sivanatarajan and Sivaramakrishnan, 2013; Adhikari, 2004; Devi, 2008; Ullah, 2014).

In the present investigation there is increase in the content of WBC ,when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. Under chemical stress of pesticides or under pathological conditions or presence of foreign substances there is increase in in WBC count due to leucocytosis in fish may be the consequence of direct stimulation of immunological defense (Marti, 1996).

Similar observation was observed i.e increase in WBC count with an increase in antibody production which helps in survival and recovery of the fish exposed to lindane and Malathion (Joshi and Deep, 2002). Various pesticides including aldrin, dieldrin, DDT, BHC, chlordane, cypermethrin, permethrin, sulfane, endosulfane, karate, endosulfan, delmethrin, diazinon,dimecron, furadon, chlordane *etc*. showed lethal effect on haematology such as changes in WBCs and RBCs,

In the present study there is increase in the content of blood glucose ,when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. Blood glucose is sensitive indicator of environmental stress for any chemical pollutant including the pescticide (Banaee ,2012)**.** The blood of fishes contains principal sugar glucose, which serve as a metabolic fuel of organs realated with level of the activity and metabolism of the animal. The blood glucose levels is maintained in

an animal , through active absorption of glucose from the digested food and also is formed from glycogen, amino acids and glycerol through glycogenolysis and gluconeogenesis under certain stress conditions.

The rise in acetyl choline levels and inhibition in the activity of acetylcholinesterase in adrenal medulla results in the rise of the content of glucose in the blood is a physiological response of the fish to meet the critical need of energy under stressed condition. Increase in the blood glucose in experimental fish compared to the control fish is , to combat the chemical stress fish produces more glucose and increase in glucose production due to increase in glucogenesis and glycogenolysis and inhibition of glycogenesis (Iwama ,1999).

Proteins is one of the most important biochemical parameters used to understand the state of fish health and biologicalmechanisms of metabolism under pollutant stress (Martinez, 2004). In the present study there is a decline in the content of total protens , when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate . Proteins are the building blocks of the body , they are important organic substances required in tissue building and repair under extreme stress conditions, protein supplies' energy in metabolic pathways and biochemical reactions (Vishwaranjan., 1988).

The reduction of protein may be due to proteolysis and increased metabolism undertoxicant stress (Remia., 2008). Similar observation were made with decrease protein content in the crab *Pratelphusa hydrodromous* in response to the pesticide Malathion toxicity (Singaraju .,1991) . In teleost fish *Oreochromis niloticus* decrease of protein content treated with glyphosate. (Shebly and Kady 2008) . A decrease in the protein level in the marine prawn *Metapenaeus monoceros* after the prawn was exposed to the pesticide methyl parathion. (Reddy and Rao 1991) Decrease in protein content in the fish *Fundulus beterolitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. (Umminger. 1970)

Due to elevation of protease activity there is a decrease in total protein and increase in free amino acids levels in both tissue and liver (Muley, 2007). Significant elevation in level of free amino acids and protease activity in *Cirrhinusmrigala* in response to cypermethrin. (Prasanth ., 2011)

Fishes have a very little amount of carbohydrates, protein are the alternative source of energy to meet the increased energy demand**.** The reduction in the content of proten in fish esposed to pescticides indicates hepatic insufficiency, high-energy demand in TCA cycle and probably malnutrition. Similar observation in decrease in protein content under toxicity stress has already been reported by several workers (Choudhary and Gaur 2001; Khare and Singh 2002; Tripathi and Singh, 2003; Kumar, 2004; Mishra, 2005; Muley, 2007; Tiwari and Singh, 2009). The decrease in total protein level and increase in free amino acids level suggest the high protein hydrolytic activity due to elevation **of** protease activity (Muley, 2007).

When *Channa punctatus* exposed to oleondrin and *Cyprinus carpio* to endosulfan a marked decrease in protein level of liver, muscle, intestine, gills and blood have been observed (Jenkins, 2003; Tiwari and Singh, 2004). Cypermethrin exposure resulted in significant decrease in protein contents in *Channa striatus* (Palanisamy and Bhaskaran, 1995), *Labeo rohita* (Veeraiah and Durga Prasad, 1998), *Tor putitora* (Ullah, 2014) and *Colisa fasciatus* (Singh, 2010).

A significant decrease in protein contents observed on exposure to Endosulfan in *Cyprinus carpio* (Jenkins, 2003; Muthukumaravel, 2013; Bibi, 2014), cypermethrin and malathion decreased protein contents in *Labeo rohita* (Thenmozhi, 2011) and *Clarias batrachus* (Khare and Singh, 2002). Thiamethoxan affected liver total protein of *Oreochromis niloticus* (Bose, 2011) while propiconazole and mancozeb induced changes in protein content in *Clarias batrachus* (Srivasatav and Singh, 2013a, c).

Amino acids are essential components of proten synthesis. In the present study there is a increase in the content free amino acid levels when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The increased levels of free aminoacid levels indicate tissue damage due to the increased proteolytic activity under chemical stress and protein mobilization by many tissues or synthesis of new proteins to adjust the cell machinery (Raja and Kulkarni , 2008) .

The increase or increase in the free amino acid content at tissue level provides valuable information during the stress condition when the fish is exposed to pesticides. Increase in concentration of free amino acids is due to the stepped up proteolysis or increase synthesis of free amino acid by transaminase reaction (James., (1979). Similar observation is made in teleost fish, *Sarotherodon mossambicus* Seshagiri Rao., (1987), However, the elevated levels of F.A.A. can be utilized for energy production by feeding them in to the TCA cycle through aminotransferase reactions.

In the present study there is significant increase in glucose levels of the tissues of when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. To combat the chemical stress fish produces more glucose and increase in glucose production due to increase in glucogenesis and glycogenolysis and inhibition of glycogenesis (Iwama ,1999).

Enzymes are biochemical molecules that control metabolic processes of organisms, thus a slight alterations in enzyme activities would adversely affect the organism (Roy, S.S., 2002). When fish is exposed to pollutants alteration in enzymes activities is observed, it is one of the major biomarker of effect of pollutants in the tissues, the organs and body fluid of the fish that can be recognized and associated with established health impairment process (Akinrotimi., 2009).

In the present study there is significant decline in SDH activity levels in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. In citric acid cycle SDH acts as a vital enzyme that catalysis the reversible oxidation of succinate to fumarate. In the present investigation it can be visualized that there is a rapid depletion of SDH activity in gill, liver and kidney tissues of fish. SDH in liver and muscle suggests higher distribution of mitochondria in these tissues, since SDH is mitochondrially localized. (Lehninger. Michael M.Cox and David L 2008 Nelson., 2006.).

The permeability of mitochondria, inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, and architectural integrity is the casue of decrease in SDH activity during pesticides stress. This prevents the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis (Shailendra Kumar Singh, Sunil Kumar Singh and Ram P. Yadav: 2010). The reduced activity of the enzyme ,succinate dehydrogenase activity in different tissues in fish *Heteropneustes fossilis* in response to dimethoate (Verma, 1980) Similar observation is recorded in the hepatopancreas and ovo testis tissues of fresh water snail *Lymnea acuminata* exposed to dimethoate and cabaryl pescticides (Tripathi and Singh , 2003)

In the present study there is significant decline in LDH activity levels in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate Lactate dehydrogenase widely distributed glycolytic enzyme occurs in present in the cells of almost all body as five different isozymes , more significantly in the metabolically active tissues and it catalyses the reversible oxidation-reduction reaction involving lactate, pyruvate, NAD+ and NADH. Lactate dehydrogenase is most sensitive enzymes to environmental pollutants. Carbohydrate metabolism involves an enzyme

lactate dehydrogenase which is used as indicative criteria of exposure to chemical stress (Diamantino TC, Amadeu E, Soaresa MVM, 2001). Decrease in the LDH activity indicates direct and indirect evidence of the cellular damage and accumulation of lactic lacid.

.

LDH is a terminal enzyme of anaerobic glycolysis, therefore, being of crucial importance to the muscular physiology, particularly in conditions of chemical stress (Coppo, J.A., N.B. Mussart and S.A. Fioranelli, 2002, Baghi, M, Hassoun, E.A and Stohs, S. J.: 1995) . The significant changes in enzymes activity of LDH indicate damage to any or all organs producing this enzyme such as liver or kidney injuries. (De Coen, W. M., Jansen, C. R., Segner, H: 1999, Young, S. J., Dowman, A and Cowell, D. C.: 2001,) LDH mediates inter-converson of lactate to pyruvate depending on the availability of co-enzyme NAD (Amacher, DE 2002)

Liver damage and change in hepatic function will increases of LDH activities (Manna PR, Eubank DW, Lalli E, Sassone-Corsi P, Stocco DM. 2003). Rise of LDH is due to the leakage of these enzymes to the blood stream (Yousef MI, Awad TI, Mohamed EH 2006). The hepatic injury might be attributed to oxidative damage by free radicals (Muthuviveganandavel V, Muthuraman P, Muthu S, Srikumar KS. 2011). Decreses in the LDH activity in tisssues under the stress of pescticide toxicant is reprted (Tripathi and Shukla , 1990, Mishra and Shukla 2003) Increased activity of LDH may specify a shift towards anaerobiosis resulting in boosted production of lactic acid (Manna PR, Eubank DW, Lalli E, Sassone-Corsi P, Stocco DM. 2003;).

In the present study there is decreasing trend of Glucose-6-Phosphatase contents in the liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate Glucose-6-Phosphatase is present in the endoplasmic reticulum of a cell. It acts as a catalyst in the conversion of glucosc-6-phosphatc to glucose. In the process of gluconeogenesis

hydrolysis of glucosc-6-phosphate is a key step. Glucose-6-phosphatase is an enzyme present in liver but not in muscle. It is a Mg+2 dependent enzyme. The chemical stress due to pescticide results in impairment of carbohydrate metabolism. For energy production via glycolosis, decarboxylation of pyruvate and citric acid cycle glucose-ephosphatase may be oxidized. Glucose-6-Phosphatase is used as a substrate for the pentose phosphate pathway yielding, both reducing power NADH, needed for the biosynthesis of fatty acids and cholesterol.

Similar observations in the alterations in the levels of glucose-6-phosphatase is observed in *Labeo rohita* exposed to fenvalerate *(* David 1995) , aldrin induced alterations in the activity of glucose-6-phosphatase in the freshwater teleost fish, observed *Channa punctatus* (Sastry .,1993) , endosulfan induced alterations in the activity of glucose-6-phosphatase in the freshwater fish, observed *Clarias batrachus* (Srinivas (1993). Diazion induced alterations in the activity of glucose-6-phosphatase observed in *Ophiocephalus* (Sastry and Sharma 1980).

Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs. This enzyme is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system (Silman I, Sussman JL, 2005., Soreq H, Seidman S. ., 2001). AChE catalyses the hydrolysis of Ach and plays a role in cholinergic transmission by reducing the concentration of acetylcholine (ACh) in the junction

to regulate nervous transmission. (Kopecka J, Rybakowas A, Barsiene J, Pempkowiak J. 2004). AChE inhibition of results in a accumulation of acetylcholine, which leads to tetany, paralysis, and eventual death due to the continuous and excessive stimulation of the nerve/muscle fibers. AChE inhibition of results to a variety of neurotoxic effects and decreased cholinergic transmission due to the build up of acetylcholine within the nerve synapses (Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT., 1998)

AChE inhibition results in functionally vital organs like gill, muscle and liver lead to impaired critical neurophysiological activity and block sodium channels of nerve filaments, thereby lengthening the depolarization phase. Pyrethroids delay the closing of the gate that allows sodium flow (Vijverberg HPM, Van den Bercken J.1990) and thus, multiple nerve impulses rather than the usual single one occur. In turn, these impulses release the neurotransmitter ACh, which stimulates other nerves (Eells JT. ., 1992) ultimately resulting in buildup of ACh within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT 1998). It is also known pyrethroid compounds which inhibit AChE activity were known to disrupt the normal behavioral patterns in the effected animals (Mushigeri SB, David ., 2005) In *Cyprinus carpio* and *Cnesterodon decemm-aculatus* showed reduce level of acetylcholinesterase (Torre ., 2002)

It has been established that the AChE enzyme unit consists of a negative subsite, which attracts the quaternary group of choline through both columbic and hydrophobic forces and an esterase subsite, where nucleophilic attack occurs on the acetyl carbon of the substrate Taylor (1980). OPs inhibitory action resulted in decrease in AChE level was probably due to the impairment of neural

transmission. The decrease in AChE activity when grass shrimp exposed to sub lethal concentration of malathion (Key. (1997), decreased AChE activity in *Prochilodus lineatus* in the brain and the muscle of when exposed to glyphosate . (Modesto and Martinez, ., 2010) .

Similar reports which collaborates our findings in the inhibition of AChE activity due to pyrethroids exposure is reported in *Cyprinus carpio (*Reddy , 1992, Szegletes., 1995) .Similar observation are made in the alterations of levels of the AChE activity in the brain, liver, muscle and gill tissues of hybrid catfish, *Clarias macrocephalus* and *Clarias gariepinus* exposed to a sublethal concentration of chlorpyrifos and a carbamate,carbaryl for 4 days and reported that AChE inhibition increased rapidly with insecticide concentration (Somnuek ., 2007) AChE inhibition is observed under cypermethrin exposure in different fishes (Rodriguez and Gold, 2000; Ahmad, 2000; Bretaud, 2000; Lionetto, 2003; Ferrari, 2007; Cattaneo, 2008; Wang, 2009; Cong, 2009; Banaee, 2011).

Proteins are the strcural and functional units of biological systems, nucleic acids play a vital role in the synthesis of proteins. The protein synthesis capacity of an organism is a index of its Nucleic acid content . Nucleic acids play a vital role in all biological activities regulate biosynthesis of proteins , growth and development . (M. Massimo, V. Milena, S. G. Scassellati, and P. Rossana, “, 2000 , G. V. Vrhovae, and D. Zeljezic, ", 2000.).

In the present study there is significant decline in DNA content in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate . Pesticides appearsas a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. In fish, DNA repairing at a much lower speed than in mammals (Walton, 1984; Espina and Wesis, 1995) The toxicant decreases the DNA content due to

inhibition of the enzymes in DNA synthesis . It is known that DNA functions as a primer in DNA and RNA polymerase reactions (Haqqi and Adhami 1979), and the inhibition in DNA content can result in the inhibition of both DNA and RNA synthesis. Alteration in nucleic acid content leads to variations in protein profile (Durai Raj and Selvarajan, 1992; Abou Donia., 1988). The process of transcription control the activities of all the enzymes , when the transcription process is curtailed, mRNA and protein synthesis does not occur, due to which metabolism is impaired.

In the present study there is significant decline in RNA content in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate The production of RNA plays a vital role in protein synthesis. The inhibition of RNA synthesis at transcription level may affect the protein content (Singh., 2010). Significant decrease of RNA observed in the presen study . Pesticides may influence directly or modify DNA ,other cellular process associated with the integrity of the genome. The pesticides interacts with the cellular DNA produces a variety of primary lesions such as single strand breaks, double strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases (Van Loon., 1991).

Similar results were also reported by various investigations: Gowri., 2013 (*Cyprinus carpio*); Veeraiah., 2013 (*Cirrhinus mrigala*); Thakur and Kakde, 2012 (*Channa punctatus*); Tiwari., 2012 (*Labeo rohita*); Singh., 2010 (*Colisa lalia*); Vasantharaja., 2013 (*Cirrhinus mrigala*); Raksheskar, 2012 (*Channa striatus*); Kumar., 2007 (*Channa punctatus*); Ansari and Kumar, 1998 (*Cyprinus carpio*); Sheela and Muniandi, 1992 (*Lepidocephalichthyes thermalis*) which substantiate the results of this study suggesting that cypermethrin is a potent inhibitor of nucleic acid synthesis even at sub lethal concentrations

Pesticides are electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway. Protein level is depleted in tissues of fish because RNA plays significant role in protein synthesis (Tripathi, G., (2002). The decrease in RNA level will affect the protein synthesis. (Maruthanayagam, C, and Sharmila, G, (2004),) reported similar results. Under stressed conditions animals require more energy to overcome the effect of pescticide. As a result, glucogenesis increases in comparsion with protein synthesis. The significant declines in RNA level in treated fish due to obstruction in RNA synthesis. Nissle bodies are rich in RNA, the swelling and chromatolysis of Nissle bodies is caused by pescticides. (Tripathi, P. K, l., (2003). Decrease in protein synthesis, impairment of nucleic acid metabolism, the degradation of cells upon exposure to pesticides may be due to, resulting in the reduction in the DNA and RNA content.

CHAPTER - 4

SUMMARY AND CONCLUSIONS

SUMMARY

Pesticides are used for controlling weeds and insect pests in agriculture and domestic uses they are easy to use and inexpensive. Pesticides include herbicides, insecticides, fungicides and more meant to kill unwanted living organisms. Most of our environment is contaminated with pesticides. Pesticide affect the non target organismsranging from beneficial soil microorganisms to insects, plants, fish, and birds.

The aim of the present study is to elucidating changes in haematological, biochemical and enzymogical responses in blood, gills liver, muscle and brain of fresh water fish *Gambusia affinis* exposed to sublethal concentration of cyfluthrin and fenvalerate for 24, 48, 72 and 96 hours .

Blood is the most important indicator of the vital physiological and biochemical functions. The quantitative flucutations in haematological parmeters like RBC, WBC, Hb, PCV and blood glucose have been observed in fresh water fish *Gambusia affinis* incontroland sub lethal concentrations of cyfluthrin and fenvalerate for 24, 48, 72 and 96 hours.

The RBC, Hb, PCV values decreased gradually in experimental fish exposed to sublethal concentration of cyfluthrin and fenvalerate due to the destruction of RBC or haemolysis or by the inhibition of erythropoesis. Where as WBC count in the experimental fish is showed a promt increase might be due to the activation of immune system to to protect the fish against the infection and cyfluthrin and fenvalerate stress.

Glucose is usable sugar found in the body fluids and tissues . Blood glucose content showed a increase in the experimental fish due to the impairement of liver to utillise glucose for glycogenolysis.

The amino acid and tissue glucose levels are increased in fish exposed to cyfluthrin and fenvalerate, where as the protein content is decreased in comparision with the control group.

The protein content of the tissues decreased in the tsiuues of experimental fish in comparision with control group due to the utilization of proteins to produce energy during chemical stress.

In the present study , the level of SDH, and LDH are decreased in gill,liver and kidney tissues of *Gambusia affinis* in experimental fish than in the control group. Decrease in the LDH activity indicates direct and indirect evidence of the cellular damage and accumulation of lactic lacid. The permeability of mitochondria, inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, and architectural integrity is the casue of decrease in SDH activity during pesticides stress.

Acetylcholinesterase activity are decreased in brain , gill,liver and kidney tissues decrease in AChE level was probably due to the impairment of neural transmission.

Glucose 6 phophatse in liver and kidney tissues decreased, for energy production via glycolosis, decarboxylation of pyruvate and citric acid cycle glucose-ephosphatase may be oxidized.

The DNA and RNA contents in tissues of gill, liver and kidney are gradually decreased in experimental fish in comparison with the control group. Decreases in the DNA content due to inhibition of the enzymes in DNA synthesis. The declines in RNA level in treated fish due to obstruction in RNA synthesis. Resulting in the swelling and chromatolysis of Nissle bodies which are rich in RNA.

CONCLUSION

It can be concluded that there is correlation between fish cyfluthrin and fenvalerate exposure and haematological, biochemical and enzymological changes observed in selected tissues. Fish blood is a valuable diagnostic tool for the investigation of disease, physiological and metabolic alterations . blood analysis , biochemicaland enzymological , nucleic acids analysis helps in understanding the effect of selected pesticides on the fsih due to the chemical stress.

In the present investigation, it is concluded that exposure to the cyfluthrin and fenvalerate results in increased oxidative stress, alternate the ani oxidant enzymes activities and morphological characters

Besides, for a safe use of this pesticide further studies should be performed to understand the exact mechanism(s) involved in the haemotological, biochemical, enzymological alterations following the cyfluthrin and fenvalerate treatment.

REFERENCES:

1. Abidi, R. and Srivastava, U.S. (1980). Effect of endosulfan on certain aspects of haematology of the fish, *Channa punctatus*. *Proc. Natl. Acad. Sci. India* 58B, 55-65.
2. Abou-Donia, M.B., Lapadul, D.M. and Carrington, C.D. 1988. Biochemical methods for assessment of neurotoxicity. In: Perspectives in basic and applied toxicology (Ed: B. Ballantyne). Butterworth & Co. Ltd., London, 1-30.
3. Adams K J, Chavasse DC, Mount DL, Carneiro IA, Curtis CF (2002) Comparative insecticidal power of three pyrethroids on netting. Med Vet Entomol 16:106–108.
4. Aderemi, F. A. (2004). Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. Tropical Journal of Animal Science, 7, 147-153.
5. Adhikari, S., Sarkar, B. (2004).Effect of Cypermethrin and Carbonfluron on certain haematological parameters and prediction of their recovery in a freshwater *teleost, Laebeo rohita* (Ham) *Ecotoxicol. Environ. Safe*. 58-220-226.
6. Affonso, E.G. (2001). Respiratory characteristics of *Hoploternum littorale* (Siluriformes, Callichthyidae); *Acta Amazonica*.31:249-262.
7. Afolabi, K. D., Akinsoyinu, A. O., Olajide, R., and Akinleye, S. B. (2010).
8. Agrawal V P and Tyagi M (1988) Haematological alterations in *Ophiocephalus punctatus* induced by endosulphan. In: *National Symposium on Threatened Habitats*, 28. Department of Zoology, Hindu College, Moradabad.

9. Ahmad S, Scopes R K, Rees G N and Patel B K C (2000) *Saccharococcus caldoxylosilyticus* sp. nov., an obligately thermophilic, xylose-utilizing, endospore-forming bacterium. *Int. J. Syst. Evol. Microbiol.* **50**, 517- 523.
10. Akinrotimi, O. A., Abu O. M. G., Ansa, E. J., Edun O. M. and George, O. S. (2009), Haematological responses of Tilapia guineensis to acute stress. J Nat Appi Sci. Vol (5):338 – 343.
11. Al-Kahtani, M.A. (2011). Effect of an Insecticide Abamectin on Some Biochemical Characteristics of Tilapia Fish (*Oreochromis Niloticus*). American Journal of Agricultural and Biological Sciences, 6 (1): 62-68.
12. Al-Makkawy, H.K., Madbouly, M.D., 1999. Persistence and accumulation of some organic insecticides in Nile water and fish. Resour. Conser. Recycl. 27, 105–115.
13. Amacher, DE**.:** A toxicologist's guide to biomarkers of hepatic response, Human and *Experimental Toxicology.,* 2002, 21, 253 -262.
14. Anandkumar A, Tripathy A P and Tripathy N K (2001). Effect of dimecron on the blood parameters of *Heteropneustes fossilis*. *J. Environ. Biol.* **22**, 297-299.
15. Anita Susan, T., K. Veeraiah and K.S. Tilak, 1999. Biochemical and enzymatic changes in the tissues of Catla catla exposed to the pyrethroid fenvalerate. J. Ecobiol., 11: 109-116.
16. Anitha Susan, T.1999. Toxicity and effect of synthetic pyrethroid fenvalerate on the metabolism of the three Indian major carps *Labeo rohita, Catla Catla* and *Cirrhinus mrigala* (Ham). Ph.D. Thesis submitted to Nagarjuna University, A.P. India.
17. Anitha Susan, T., Veeriah, K and Tilak, K.S. (1999): Biochemical and enzymatic changes in the tissues of Catla catla exposed to the pyrethroid fenvalerate. J. Ecobiol., 11(2): 109-116.

18. Annamani AK (1986). Toxicity of Fenvalerate technical grade and 20% EC to *Labeo rohita* and *Aplochelus panchax*. M.Phil. Thesis, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.
19. Ansari, B.A. and Kumar, K. 1988. Cypermethrin Toxicity: Effect on the carbohydrate metabolism of the Indiancatfish, *Hetropneustes fossilis* – Sci. Total Environ., 72:161-166.
20. APHA. 1998. Standard Methods for the examination of water and waste water. (20th edn.), American Public Health Association, New York.
21. Apostol S. The toxicity of certain pesticides to protozoa. Proc Symp Environ Biol 1979 : 97-102.
22. Asztolas, B., Nemasok,J.,Benedeezky Gabrial, R, Szabo, A and Refarieo, J. (1990): The effect of pesticides on some biochemical parameters of carp C. carpio. Arch. Environ. Contam.Toxicol., 19: 257-280.
23. Babu velmurugan, Mariadoss selvanayagam, Elif I. Cengiz and Erhan Unlu: The Effects of Fenvalerate on different tissues of fresh water fish Cirrhinus mrigala. J. Environ. Sci. Hlth., Part-B, 42, 157-163 (2007).
24. Baghi, M, Hassoun, E.A and Stohs, S. J.: *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, 1995,104, 129-140.
25. Baird and Girard, 1853.Global Invasive Species Database (GISD) 2012. Species profile: *Gambusia affinis* .
26. Banaee, M. Mirvagefei, A. R. Rafei, G. R. & Majazi Amiri, B. (2008). Effect of sub-lethal Diazinon Concentrations on Blood Plasma Biochemistry. Int. J. Environ. Res., 2(2): 189-198.
27. Banaee M. (2012). Adverse effect of insecticides on various aspects of fish's biology and physiology: Insecticides – Basic and Other Applications Book, Edited by Sonia Soloneski and Marcelo Larramendy, Published by InTech, Chapter, 6: 101-126.

28. Bansal, M. L., Singh, S., Bansal, M. L., Singh, T. and Kumar, R. (2004).Statistical methods for Research Workers.Kalyani publishers, New Delhi-2.
29. Bansal S K, Verma S R, Gupta A K and Dalella R C (1979) Physiological dysfunction of the haemopoietic system in afreshwater teleost, *Labeo rohita* following chronic chlordane exposure. Part 2. Alterations in certain organic compounds and serum electrolytes. *Bull. Environ. Contam. Toxicol*. 22, 674- 680.
30. Barcellos L.J.G, Kreutz L.C, Rodrigues L.B, Fioreze I, Quevedo R.M, Cericato L, Conrad J, Soso A.B, Fagundes M, Lacerda L.A, and Terra S. (2003). Haematological and biochemical characteristics of male jundiá (RhamdiaQuelen, Quoy andGaimaRDT, Pimelodidae): changes after acute stress. Aquaculture Research. 34: 1465–1469.
31. Bauman, F. (1981). Draft environmental report of India. Saota 1-77074 PN-AAH752 Arda 2:31-32.
32. Benli, A.C.K., 2005. Investigation of acute toxicity of cyfluthrin on tilapia fry (Oreochromis niloticus L. 1758). Environ. Toxicol. Pharmacol. 20, 279–282.
33. Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P. and Wahli, T. (1999). Histopathology in Fish: proposal protocol to assess aquatic pollution. *J. Fish Dis*., 22: 25-34.
34. Benli, A.C.K. (2005) Investigation of acute toxicity of cyfluthrin on tilapia fry (*Oreochromis niloticus* L. 1758). *Environmental Toxicology and Pharmacology* 20: 279-282.
35. Bhagwat, R.P. and Banerjee, V. (1986). Haematology of an Indian freshwater Eel *Amphipnous cuchia* (Hamilton) Erythrocyte counts related parameters with special reference to body length sex and season. *Compt. physiol. Ecol*. 2(1); 21-27.

36. Bhatnagar MC, Bana AK. Respiratory distress to clarias batrachus exposed to endosulfan-a histological approach. J. Environ. Biol. 13, 1992, 227-231.

37. Bhatkar N V and Dhande R R (2000) Furadon induced haematological changes in the freshwater fish, *Labeo rohita*. *J. Ecotoxicol. Environ. Monit*. **10**, 193-196.

38. Bhatia N P, Sandhu G S and Johal M S (2002) Endosulfan induced changes in blood chemistry of *Heteropneustes fossilis*. *Poll. Res*. 21, 323-327.

39. Bhatia N P, Sandhu C S and Johal M S (2004). Haematological alterations in *Heteropneustes fossilis* upon exposure to endosulfan. *Poll. Res.* 23, 633-636.

40. Bibi N, Zuberi A, Naeem M, Ullah I, Sarwar H and Atika B (2014) Evaluation of acute toxicity of karate and its sub-lethal effects on protein and acetylcholinesterase activity in *Cyprinus carpio*. *Int. J. Agri. Biol.* **16**, 731-737.

41. Binu Kumari, S. and Subisha, M.C. (2010). Haematological responses in a fresh water fish *Oreochromis mossambicus* exposed to Chloripyrifos.The ekol.10 (1-2) 83-88.

42. Bose S, Nath S and Sahana S S (2011) Toxic impact of thiamethoxam on the growth performance and liver protein concentration of a freshwater fish, *Oreochromis niloticus* (Trewavas). *Indian J.Fundam. App. Life Sci.* **1**, 274-280.

43. Bowes, C.V. 1972. Uptake and metabolism of 2,2 bis (P. Chlorophenyl) 1-l,ltrichloroethane (DDT) by marine phytoplankton and its effects on growth and chloroplast electron transport. *Plant Physiology* 49: 72-116.

44. Bradbury, S.P., Coats, J.R., 1989. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contam. Toxicol. 108, 133–177.

45. Bradbury S.P., Coats J.R. and Mckim J.M., 1985. Differential toxicity and uptake of two fenvalerate formulations in fathead minnows (Pimephales promelas). Environ. Toxicol Chem., 4:533-541.

46. Bretaud S, Toutant J P and Saglio P (2000) Effects of carbofuran, diuron and nicosulfuran on acetylcholinestrase activity on goldfish (*Carassius auratus*). *Ecotoxicol. Environ. Saf.* **47**, 117- 124.

47. Cappon ID, Nicholas DM (1975). Factors involved in increased protein synthesis in liver microsomes after administration of DDT. Pestic. Biochem. Physiol., 5: 109-118.

48. Cattaneo R, Loro V L, Spanevello R S, Moraes B S and Clasen B (2008) Metabolic and histological parameters of silver catfish(*Rhamid quelen*) exposed to commercial formulation of 2, 4- dichlorophenoxi-acetic acid (2, 4-D) herbicide, *Pestic. Biochem. Physiol.* **92**, 133-137.

49. Casida JE, Benett JP, Vickery BH, Gammon DW(1983): Mechanism of selective action of pyrethroid insecticide. Annual Review of Pharmacology and Toxicology, 23, 413-438.

50. Carpentor, P.L. (1975): In: Immunology and Serology. 3rd eds. W.B. Saunders Company. Philadelphia. London. Toronto. P.254.

51. Caroll, N.V., Longley, R.W and Rose, J.H. (1956): Glycogen determination in liver and muscle by use of anthrone reagent. J. Biol. Chem., 220: 583-593.

52. Chapman, R.A. and Harris, C.R. 1981. Persistance of four pyrethroid insecticides in sterile and natural, mineral and organic soil, *Bull. Environ. Contom. Toxicol,* 20: 513-519.

53. Chapman, R.A., Tu, C.M., Harris, C.R and Cole, C. 1981. Persistence of five pyrethroid insecticides on Rainbow trout. *Bull. Environ. Contam. Toxicol. 26 :* 513-519.

54. Chauhan, R., Saxena, K. and Kumar, S. (1994).Rogor extract of the bark of Buchanian lanzanlin on behavior and chromatophores of freshwater fish *Labeo rohita*; *J. Envion. Biol.* 22(3):229-231.

55. Chindah AC, Sikoki FD, Vincent-Akpu I: Changes in haematological characteristics of a bony fish, Tilapia guineensis (Bleeker, 1862), exposed to common pesticides in the Niger Delta Wetland, Nigeria, Ghana Journal of Agricultural Science 2004; 37: 59-67.

56. COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency. Ecol. Res. Ser. Publ. No. EPA-660/3-75-009. 61 p.

57. Cong N V, Phuong N T and Bayley M (2009) Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). *Ecotoxicol. Environ. Saf.* **72**, 699-703.

58. Coppo, J.A., N.B. Mussart and S.A. Fioranelli: Physiological varaiation of enzymatic activities in blood of Bullfrog, *Rana catesbeina* (Shaw, 1802). *Rev.Vet.,* 2002,12/13, 22- 27.

59. Cremlyn, R.: 1978, *Pesticides: Preparation and Mode of Action*, pp. 50-130, John Wiley and Sons, New York.

60. Dalela, R.C., Ravi, S., Kumar, V and Verma, S.R. (1981): In vivo haematalogical alternations in a fresh water teleost Mystus following sub acute exposure to pesticides and their combinations. J. Environ. Biol., 2 : 79-86.

61. Das B.K., Mukherjee S.C. (2003). Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences. Comparative Biochemistry and Physiology, Part C, 134: 109–121.

62. Dhasarathan, P., Palanlappan, R. and Singh, A. (2000). Effect of Endosulfan and Butachlor on the digestive an enzyme proximate composition of the fish *Cyprinus carpio*. *Indian J.Environ* and *Ecoplan* 3(e): 339-446.

63. David,M., Shivakumar, R Mushegeri, S.B. and Kuri, R.C. (2005): Blood glucose and glycogen levels as indicator of stress in the fresh water fish

Labeo rohita under fenvalerate intoxication. J. Ecotoxicol. Environ. Monit., 15(1): 01-05.

64. David, 1985, Preventive measures more beneficial than pesticides, The Hindu, 24th September, 1985.
65. David, M., (1995), Effect of fenvalerate on behavioural, physiological and biochemical aspects of fresh water fish, Labeo rohita (Hamilton). Ph.D. Thesis, S.K. University, Anantapur.
66. Davidson I. and J.B. Henry (1969) : Todd-sanford clinical diagnosis by laboratory methods. 14th Ed. W.B. Sauders Co., Philadelphia, London, Toronto, 139-143.
67. De Coen, W. M., Jansen, C. R., Segner, H: The use of biomarkers in *Daphnia magna* toxicity testing V. *In vivo* alternations in the carbohydrate metabolism of *Daphnia magna* exposed to sublethal concentrations of mercury and lindane. *Ecotoxicol. Environ. Safety.*, 2001, 48, 223-234.
68. Demoute JP (1989) A brief review of the environmental fate and metabolism of pyrethroids. Pestic Sci 27:375–385.
69. Devi P, Baruah D, Baruah B K and Borkotoki A (2008) Impact of endosulfan on some haematological parameters of *Channa punctatus* (Bloch). *Poll. Res*. **27**, 485-488.
70. Dezwaan A, Zandee DT (1972). The utilization of glycogen accumulation of some intermediates during anaerobiosis in *Mytilus edulis L*. Comp. Biochem. Physiol. B: 43: 47-54.
71. Diamantino TC, Amadeu E, Soaresa MVM, Guilherminoc L. Lactate dehydrogenase activity as an effect criterion intoxicity tests with *Daphnia magna straus*. Chemosphere 2001; 45:553-560.
72. Di Giulio R.T. and Hinton D.E. (2008). The Toxicology of Fishes. CRC Press USA.

73. Durairaj, S. and Selvarajan, V.R.1992. Influence of quinalphos, an Organophosphorous Pesticide, on the Biochemical constituents of the tissues of fish, Oreochromis mossambicus J.Environ. Biol. 13(3): p. 181-185.
74. Eells JT. Pyrethroid insecticide induced alterations in mammalian synaptic membrane potential. Pharmacol Exper Therap 1992; 262:1173-1181.
75. Edwards CA (1973). Environmental Pollution by Pesticides. Plenum Press, London, New York Ghosh TK, Konar SK. Effects of sublethal levels of the insecticide aldrin on fish and fish-food organisms. Proc Symp Environ Biol 1979;103-112.
76. Ellman, G.L., Courtney, K.D., Andre, J.V. and Featherstone, R.M. (1961) A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochemical Pharmacology, 7, 88-95.
77. EPA Pesticide Fact Sheet: Cyfluthrin. 1987. Fact Sheet No. 164 EXTOXNET. 2001. Cyfluthrin. Available online http://ace.orst.edu/cgi-bin/mfs/01/pips/cyfluthr.html.
78. Espina N G and Wesis P (1995) DNA repair in fish from polluted estuaries. *Mar. Environ. Res.* 39, 309-312.
79. Farm Chemical Handbook. 1993. Meister Publishing Company. Vol. 79.
80. Florkin M, Schoffenials E (1964). In: Studies in Comparative Biochemistry. (Manday KA ed.) Mac Milan, Pergamon press, New York.
81. Ferrari A, Venturino A D and Anglo A M (2007) Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Onchorhyncus mykiss*) detoxifying enzymes. *Insect. Biochem. Physiol*. **88**, 134- 14.
82. Finney DT. Probit Analysis, 3rd edit. Cambridge University Press. London, 1971

83. Froese, R., and D. Pauly, editors. 2016. Gambusia affinis (Baird & Girard, 1853). FishBase. Available: http://www.fishbase.us/summary/Gambusia-affinis.html. (May 2016).

84. Gautam R K and Kumar S (2008) Alteration in haematology of *Channa punctatus* (Bloch). *J. Exp. Zool. India*. 11, 309-310.

85. Ghosh, T.K., 1986. Effect of Dimethoate on tissue glycogen content of some freshwater fish. Env. Ecol., 4: 554-557.

86. Gill, T.S., Pande, J. and Tiwari, H. (1992). Effect of Endosulfan on the blood and organ chemistry of freshwater fish, *Barbus conchonitus* (Ham). *Ecotoxicol. Environ.Saf*.21:80-89.

87. Govindappa, S. and Swami, K.S. (1965). Electrophoretic characteristics of subcellular components and therir relation to enzyme activities in subcellular components and their relation to enzyme activities in amphibian muscle fibres. Indian. J. Exp. Biol. 3(4): 203 – 212.

88. Gowri, B., Govindassamy, P. and Ramalingam, V. 2013. Influence of Cypermethrin on DNA and RNA Content in Different Organs of Freshwater Fish *Cyprinus carpio*. Iran. J. Pharm. Sci., 9(3): 1-10.

89. Gupta, P.K. and Satankhe, D.K. 1985. Modem Toxicology Vol. II. The adverse effect of Zenobiotics. Metropolition book company Private Ltd., New Delhi. 15-17.

90. Haqqi, T.M. and Adhami, U.M. 1979. Effect of apholate (ENT 26216, NSC 26812) on nucleic acids, total protein content and activity levels of aminotransferases (GOT, GPT), alkaline phosphatase and 5-nucleotidase in liver of male albino rats. Ind. J. Exp. Biol., 17: 488- 492.

91. Hamman, I. And R. Fuchs. 1981. ®Baythroid, a new insecticide. Pflanzenschutz- Nachrichten, 34(2):121-151.

92. Harper JW (1985). In: Harpers Review of Biochemistry (eds) Martin DW, Mayes PA, Rodwell VW. Lange Medical Publications, Maruzen Asia

(Singapore). Haya K (1989). Toxicity of pyrethroid insecticides to Fish Environ. Toxicol. Chem., 8: 381-391.

93. Haya, K. 1989. Toxicity of pyrethroid insecticides to fish. Environ. Toxicol and Chem. Vol.8: 381 –391.

94. Hazarika, R. and Das, M. 1998. Toxicological impact of BHC on the ovary of the air-breathing catfish *Heteropneustes fossilis* (Bloch). *Bulletin of Environmental Contamination and Toxicology,* 60: 16-21.

95. Hill, I. R, (1985), Taylor & Francis. London. Pg 165–181.1

96. Hingorani, G.H. Madhusudhana Rao, K. and Ramachandra, R. 1973. An attempt to study pollution and fish mortality in Hussainsagar reservoir in Hyderabad. *J. Ind. Fish. Ass., 3 :* pp. 232.

97. Holden, A.V. 1965. A study of the absorption of Cm labelled DDT from fresh water fish^nn. *Appl. Biol.* 50: 467-477.

98. Israel Stalin, S and Sam Manohar Das, S. (2012): Biochemical changes in certain tissues of Cirrhina mrigala (Hamilton) exposed to fenthion. Int. J. Environ. Sci., Vol:2, No;3.

99. Iwama, G. K., M. M. Vijayan, R. B. Forsyth and P. A. Ackerman. 1999. Heat shock proteins and physiological stress in fish. Am. Zool. 39:901-909.

100. Jacob, S. S., Balakrishnan, Nair N. and Balasubramanian, N. K. 1982. Toxicity of certain mosquito larvicides to the larvivorous fishes Aphocheilus lineatus (Cuv. and Val.) and Macropodus cupanus (Cuv. and Val.). Environ. Pollut. 28: 7-13.

101. Jagan, P., Reddy, M. S. and Rao, A. P. 1989. Effect of certain insecticides on the freshwater fish *Cyprinus carpio carpio*, L. *Indian Journal of Environmental Biology,* 10 (2): 135-138.

102. James JH, Ziparo V, Jeppsson B, Fischer JE (1979). Hyperammonemia, plasma amino acid imbalance and blood brain aminoacid transport: A unified theory of portal systemic encephalopathy. Lancet, 2: 772-775.

103. Jenkins F S, Rajanna B J, Shameem U and Mahadevi R (2003) Effects of sublethal concentration of endosulfan on hematological and serum biochemical parameters in the carp, *Cyprinus carpio*. *Bull. Environ. Contam.Toxicol*. 70, 993-997.

104. Joshi P and Deep H (2002) Effect of lindane and malathion exposure to certain blood parameters in a freshwater teleost fish, *Clarias batrachus*. *Poll. Res*. 21, 55-57.

105. Johal M S and Grewal H (2004) Toxicological study on the blood of *Channa punctatus* (Bloch) upon exposure to carbaryl. *Poll. Res.* 23, 601-606.

106. Joshi P K Pandey A K and Nagar R K (1988) Electrophoretic variation in the blood protein fractions in malathoin treated fish, *Channa puncatus* (Bloch). In: *Proceedings of the National Symposium on Animal Metabolism amd Pollution*. 147-149.

107. Joia *et ah,* 1979. As cited by Nanda Kumar, 1982. S.V. University, Tirupati on environmental monitoring of Pesticide residues in some districts of Andhra Pradesh.

108. Kabeer Ahamed SI, Sambasiva Rao KRS, Ramana Rao KV (1983). Dehydrogenase systems of the teleost *T. mossambica* under augmented sublethal malathion stress. J. Anim. Morphol. Physiol., 30 (1&2).

109. Kajare, A., S. Singh and K. Shrivastava, 2000. Malathion induced biochemical changes in the kidney of freshwater fish Clarias batrachus. J. Ecotoxicol. Environ. Monit., 10: 11-14.

110. Kale, M.N., S. John Rathore and D. Bhatnagar, 1999. Lipid peroxidative damage on pyrethroids exposure and alternation in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol. Lett., 105: 197-205.

111. Kemp, A., Adrinene, J.M., Kits and Van Hejnenga, A colorimetric method for the determination of glycogen in tissues. J. Biol. Chem., 1954, 56: 646-648.

112. Key, P.B., M.H. Fulton, G.I Scott, S.L. Layman E.F. Wirth, 1998. Lethal and sublethal effects of Malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. Aquatic Toxicol. 40: 311-322.

113. Khan, T. A., and Zafar, F. (2005). Haematological Study in Response to Varying Doses of Estrogen in Broiler Chicken. International Journal of Poultry Science, 4(10): 748-751.

114. Khare A and Singh S (2002) Impact of malathon on protein content in the freshwater fish, *Clarias batrachus*. *J. Ecotoxicol. Environ.Monit*. 12, 129-132.

115. Kim, J., Chehade, J., Pinnas, J.L., Arshag, D., Mooradian, A.D., 2000. Effect of select antioxidants on malondialdehyde modification of proteins. Nutrition 16, 1079–1081.

116. Kopecka J, Rybakowas A, Barsiene J, Pempkowiak J.AChE levels in mussels and fish collected off Lithuania and Poland (Southern Baltic). Oceanologia 2004; 46:405- 418.

117. Koundinya PR (1979). Studies on physiological responses of teleost, *T. mossambica* to pesticide impact. Ph.D. thesis, S.V. University, Tirupati, India.

118. Kumar, A., Sharma, B. and Pandey, R.S. 2007. Preliminary evaluation of the acute toxicity of cypermethrin and λ- cyhalothrin to *Channa punctatus*. Bull. Environ. Contam. Toxicol., 79: 613- 616.

119. Kumar K, Patri P and Pandey A K (2004) Haematological and biochemical responses in the freshwater air-breathing teleost, *Anabas testudineus* (Bloch) exposed to mercury. *J. Ecophysiol. Occup. Hlth.* 4, 97-108.

120. Kumar K, Sinha Y K P and Pandey A K (1999a) Endosulfan induced hematotogical alterations in the climbing perch, *Anabas testudinenus* (Bloch). *J. Natcon.* **11**, 125-134.

121. Kumar S, Singh M (2000). Toxicity of dimethoate to a fresh water teleost *Catla catla*. J. Exp. Zool., India, 3(1): 83-88.

122. Lacerda ,T.P and Swaya, P. (1986): Effect of two hypo osmotic conditions on haemolymph glucose concentration in Callinectes danae(Crustacea). Comp. Biochem. Physiol., 85(2): 509-512.

123. Lakshmi Rajyam Ch (1989). Evaluation of toxic effects of a synthetic pyrethroid fenvalerate in albino rat. Ph.D. thesis, S.V. University, Tirupati, India.

124. Latner, A.L., 1975. In: Clinical biochemistry (Ed.: Contarow and Trumper). Saunders Company, Philadelphia, pp: 182-192.

125. Leahey, J.P. (ed.) (1985) The Pyrethroid Insecticides. Taylor and Francis, London and Philadelphia.

126. Lehninger AL (1983). In: *Biochemistry*, Kalyani Publications and Ludhiana, New Delhi.

127. Lehninger principles of biochemistry 5th edition. Michael M.Cox and David L Nelson. New York, 2008**.**

128. Lionetto M G R, Caricato M E, Giordano M F and Schettino T (2003) Integrated use of biomarkers(acetylcholineestrase and antioxidant enzymes activities) in Mytilus *galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. *Mar. Pollut. Bull*. 46, 324-330.

129. Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.

130. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J., Protein measurement with folin phenol reagent, J. Biol. Chem.,1951, 193: 265-275.

131. Luther Das, P.N., Jayaprada, P and Veeraiah, K. (1999): Toxicity and effect of cypermethrin on biochemical constituents of fresh water teleost Channa punctatus. J. Ecotoxicol. Monit., 9(30): 197-203.

132. Lynch, M.J., Raphael, S.S., Mellor, L.D., Spare, P.D. and Inwood, M.J.H.1969. Medical laboratory technology reagent J. Biol. Chem. 193. P. 265-275.

133. Macek, K.J; Hutchinson, C. and Cope, C.B. 1969. Effect of temperature on the susceptibility of blue gills and rainbow trout to selected pesticides. *Bull. Environ. Contam. Toxicol. 4.* 174.

134. Madan, V.K., Singh, R., Kumari, B., Naresh, J.S., Kathpal, T.S. 2000. "Dissipation of lindane and fenvalerate residues in chickpea (Cicer arietinum L.) under Indian climatic conditions". Ecotoxicology and Environmental Safety. 46: 163-166.

135. Majumdar, T.N. and A. Gupta: Acute toxicity of endosulfan and malathion on Chironomus ramosus (Insecta : Diptera : Chironomidae) from north Cachar hills, Assam, India. J. Environ. Biol., 30, 469-470 (2009).

136. Majumder and Krishnamurthy, 1963. As cited by N.V. Nanda Kumar 1982. S.V.University, Tirupati on Environmental monitoring of pesticide residues in some districts of Andhra Pradesh.

137. Malla Reddy P, Bashamohideen Md. (1988). Toxic impact of fenvalerate on the protein metabolism in the branchial tissue of the fish, *Cyprinus carpio*. Curr. Sci., 57: 211-212.

138. Manna PR, Eubank DW, Lalli E, Sassone-Corsi P, Stocco DM. Transcriptional regulation of the mouse steroidogenic acute regulatory protein gene by the Camp response-element binding protein and steroidogenic factor 1. Mol Endocrinol 2003; 30:381-397.

139. M. Massimo, V. Milena, S. G. Scassellati, and P. Rossana, "Pesticide induced primary DNA damage in peripheral blood leucocytes of farm

workers evaluated by the computerized comet assay", Biomarkers, 5, 192-204, 2000.

140. Marti H H, Wenger R H and Rivas L A (1996) Erythropoietin gene expression in human, monkey and murine brain. *Euro. J. Neurosci*.8, 666-676.

141. MARTINEZ, C. B., M. Y. NAGAE, C. ZAIA, AND D. A. M. ZAIA. 2004. Acute morphological and physiological effects of lead in the neotropical fish Prochilodus lineatus. Brazilian Journal of Biology 65:797–807.

142. Maruthanayagam, C, and Sharmila, G, (2004),Nature Env. Polln. Techn. Vol-3, issue 4, pg 491–494.

143. Mathivanan R (2004). Effects of sublethal concentration of quinalphos on selected respiratory and biochemical parameters in the freshwater fish, *Oreochromis mossambicus*. J. Ecotoxicol. Environ. Monit., 14: 57-64.

144. Mathur , S.C. 1999. Future of Indian pesticides industry in next millennium. Pesticide Information. 24(4): 9-23.

145. Meister, R.T. (1995) Farm Chemicals Handbook '95. Meister Publishing Company. Willoughby, OH.

146. Mishra D K, Bohidar K and Pandey A K (2005) Effect of sublethal carbary and cartap exposure on protein metabolism of the teleost, *Channa punctatus* (Bloch). *J. Inland Fish. Soc. India* 37, 30-34.

147. Mishra, R. and S. P. Shukla: Endosulfan effects on muscle malate dehydrogenase of the freshwater catfish, Clarias batrachus. Ecotox. Environ. Safe., 56, 425-433 (2003).

148. Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT. Common mechanism of toxicity: A case study of organophosphorus pesticides. Toxicol Sci 1998; 41:8-20.

149. MIURA, T. & TAKAHASHI, R.M. (1976) Effects of a synthetic pyrethroid, SD43775, on nontarget organisms when utilized as a mosquito larvicide. *Mosq. News,* 36: 322-326.

150. Miyamoto J (1976): Degradation metabolism and toxicity of synthetic pyrethroid. Environmental Health Perspectives, 14,15-28.

151. Moore, S. and Stein, W.H. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J.Biol.Chem., 211: 907 – 913.

152. Modesto, K.A, Martinez, C.B.R. 2010. Effects of Roundup Transorb on fish: hematology, antioxidant defences and acetylcholinesterase activity. Chemosphere 81: 781-787.

153. Mukherjee Kanual, L. (1988) Medical Laboratory Technology (Edn).Volume-1, Tata, Mc Graw Hill Publishing Company Limited, New Delhi.

154. Muley DY, Mane UH. Toxicity of endosulfan 35 EC to estuarine edible clam Katelysia opima (Gmelin) from west coast of India. Proc Natl Symp Environ Biol 1986;117-123.

155. Muley D V, Karanjkar D M and Maske S V (2007) Impact of industrial effluents on the biochemical composition of freshwater fish, *Labeo rohita*. *J. Environ. Biol*. 28, 245-249.

156. MULLA, M.S., NAVVAB-GOJRATI, H.A., & DARWAZEH, H.A. (1978a) Toxicity of mosquito larvicidal pyrethroids to four species of freshwater fishes. *Environ. Entomol.*, 7: 428-430.

157. MULLA, M.S., DARWAZEH, H.A., & DHILLON, M.S. (1980) New pyrethroidsas mosquito larvicides and their effects on nontarget organisms. *Mosq. News,* 40: 6-12.

158. Müller, (1946), Über Zusammenhänge zwischen Konstitution und insektizider Wirkung, **29**, Genf / Basel: Helv. Chim. Acta, pp. 1560–1580.

159. Murphy, S.D. 1980. Pesticides in : Toxicology the basic science of poisons, P.357. In. Amdur, M.D, (eds) Me Millan Publishing Co. Inc. Newyork.

160. Murty and Priyamvada Devi. 1981. Reprint from: Bull. Environ. Coutam. Toxicol.27: 239-243.

161. Mushigeri SB, David M. Fenvalerate induced changes in the ACh and associated AChE activity in different tissues of fish, *Cirrhinus mrigala* (Hamilton) under lethal and sublethal exposure period. Env Toxicol Pharmacol 2005; 20:65-**72.**

162. Mustak, M. and Natarajan, K. (1992). Altered physiological parameters due to the pesticide Ekalux in the fish *Catla catla*. *Environ and Ecol*., 10 (2): 445-447.

163. Muthukumaravel, K and Murthy, A. (2009): Blood glucose level and hepatic glycogen interrelationships in Labeo rohita exposed to heavy metal chromium. J. Ecobiol., 25(4):307-310.

164. Muthukumaravel K, Sivakumar B, Kumarasamy P and Govindarajan M (2013) Studies on the toxicity of pesticide monocrotophos on the biochemical constituents of the freshwater fish, *Labeo rohita*. *Int. J. Curr. Biochem. Biotechnol*. 2 (10), 20-26.

165. Muthuviveganandavel V, Muthuraman P, Muthu S, Srikumar KS. Individual and combined biochemical and histological effect of cypermethrin and carbendazim in male albino rats. Appl Pharma Sci 2011; 1:121-129.

166. Nachlas, M.N., Margulus, S.I, and Seligman, A.M. (1960). A colorimetric method for the estimation of succinic dehydrogenase activity. J. Biol. Chem., 235: 499 – 503.

167. Nakano, T and Tomlinson, N. (1967): Catecholamines and carbohydrate concentrations in rain bow trout S. gairdeneri in relation to physical disturbance. J. Fish. Res. Bd. Canada. 24: 1701-1715.

168. Narahashi, T. 1983. Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. Pestic. Biochem. Physiol., 20 : 203-216.

169. Natarajan GM (1983). Metasystox toxicity: Effects of Lethal LC50 / 48 hrs concentration on free aminoacids and glutamate dehydrogenase in some tissues in the air breathing fish, *Channa striatus* (Bleeker). Comp. Physiol. Ecol., 8: 254-256.

170. National Institute of Nutrition, 1977. An Annual report by National Institute of Nutrition, Hyderabad, Andhra Pradesh.

171. Nelson, J. 2006. Fishes of the world 4th edition. John Wiley & Sons Inc., Hoboken, New Jersey.

172. Nelson, N. and Somogyi , M.1952.A photometric adaptation of Somogyi method for the determination of glucose . J.Biolo. Chem. 151:375-380.

173. O'Brien, R.D. (1967). Insecticides action and metabolism. NeuYork Academic. Press.

174. Odum, E.P., 1971. Fundamentals of Ecology (W.B.Saunders), Toppanlo, Ltd., Tokyo, Japan.

175. Olafedehan, C. O., Obun, A. M., Yusuf, M. K., Adewumi, O. O., Oladefedehan, A. O., Awofolaji, A. O., andAdeniji, A. A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits. Proceedings of 35th Annual Conference of Nigerian Society for Animal Production, 212-218.

176. Omprakasam M, Dayalan A, Arul Ganesan M (2008). Aquatic toxicity of dimethyl sulfoxide on the Indian major carp, *Catla catla* (Hamilton). Indian J. Sci. Technol., 1(4): 1-4.

177. Öztürk, S., Ikiz, R., (2003). Akgöl (Fethiye- Mugla) Sivrisinek Baligi *Gambusia affinis* (Baird&Girard, 1853) Populasyonunun Bazi Biyolojik Özellikleri, *Turkish Journal* of *Veterinary* and *Animal Sciences*, 27: 911- 915. Page,L.M., Burr, B.M., (1991). A field guide to freshwater fishes of North America north of Mexico, Houghton Mifflin Company, Boston.

178. Rayms-Keller, A., Olson, K.E., McGaw, M., Oray, C., Carlson, J.O. and Beaty, B.J., (1998). Effect of Heavy Metals on *Aedes aegypti* (Diptera:Culicidea) Larvae, E*cotoxicology and Environmental Safety*. 39:41-47. doi:10.1006/eesa.1997.1605.

179. Palanisamy S and Bhaskaran P (1995) Selected biochemical and physiological response of the fish *Channa striatus* as biomonitorto assess heavy metal pollution in freshwater environment. *J. Ecotoxicol. Environ. Monit.* 5, 131-138.

180. Pant, J., Tewari, H and Gill, T. (1987): Effect of aldicarb on the blood and tissues of fresh water fish Barbus conchonius. Bull. Environ. Contam. Toxicol., 38: 36.

181. Pandey, A.C., Pandey, A.K and Das, P. (2000): Fish and fisheries in relation to aquatic pollution In: Environmental Issues and management. S.R. Verma, A.K. Gupta and P. Das 9(Eds) Nature Conservators. Muzaffarnagar. P.87-112.

182. Pandi B (1991). Use of Biochemical parameters in biomonitoring of pesticide pollution in some fresh water fishes: J. Ecotoxicol. Environ. Monitoring, 2: 101-104.

183. Patole, S. S. and Bhoi, S.S. (2013). Comparative studies on haematological responses in the fresh water fish, *Punctius conchonius* exposed to two piscicidal plants extracts.

184. Pickering, Q.H. and Henderson, C. 1966. The acute toxicity of some pesticides to fish. Ohio. J. Sci., 66(5): 508 -513.

185. Potts WTH (1958). The inorganic and aminoacid composition of somelamellibranch mussels. J. Exp. Biol., 35: 749-764.

186. Pramoda Kumari J, Naidu BP (1987). Effect of permethrin on the organic constituents of the silk worm *Bombyx mori*. Environ. Ecol., 5: 717-719.

187. Prashanth M S, Sayeswara H A and Goudar M A (2011) Free cyanide induced physiological changes in the freshwater fish, *Poecilia reticulata*. *J. Exp. Sci*. 2 (2), 27-31.

188. Radhaiah V (1988). Studies on the toxic impact of a pyrethroid insecticide fenvalerate on some metabolic aspects and histopathology of a fresh water teleost *Tilapia mossambica* (Peters). Ph.D. thesis, S.V. University, Tirupati, India.

189. Radaiah, V and Jayantha Rao, K. (1990): Toxicity of pyrethroid insecticide fenvalerate to a fresh water fish T. mossambica (Peters). Ecotoxicol. Environment . Saf., 20(1): 117-124.

190. Raja ,I.A and K.M.Kulkarni ,2008.Tissue biochemical adjustments in the air breathing fresh water fish *Channa punctatus* exposed to environmental hypoxia .*Environ. Ecol*. 26(2): 669-672.

191. Raksheskar, G.A. 2012. Influence of cypermethrin on DNA, RNA and RNA/DNA ratio in gills of the freshwater fish Channa striatus. Biosci. Disc., 3: 17-19.

192. Ramana Kumari. G. 1999. Toxicity and effect of chloropyrifos on Indian major carp *Labeo rohita.* M.Phil. Dissertation submitted to Nagarjuna University, Guntur, A.P. India.

193. Ramaneswari, K. and L.M. Rao: Influence of endosulfan and monocrotophos exposure on the activity of NADPH cytochrome C reductase (NCCR) of Labeo rohita (Ham). J. Environ. Biol., 29, 183-185 (2008).

194. Ramesh, M. and Saravanan, M. (2008). Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to Chloripyrifos; *Int. J. Inter. Biol*. 3 (1):80-83.

195. Rao, D.M.R. 1989. Studies on the relative toxicity and metabolism of endosulphan to the Indian Major carp Catla catla with special reference to some biochemical changes induced by the pesticide. Pestic. Biochem. Physiol. 33: 220-22 .

196. Rathod, D.S., M.V. Lokhande and V.S. Shembekar, 2009. Toxic impact of the biochemical composition of vital tissues of fish, Arias dussumieri. Shodh, Samiksha and Mulyankan, 2: 147-149.

197. Reddy, P.S and K.V.R. Rao, 1991. Methyl parathion induced alternations in the tissuecarbohydrate catabolism of marine prawn *Metapenaeus monoceros*. Bull. Environ. Contam. Toxicol., 47: 925-932.

198. Reddy, M.M., G.H.Philip and Md.Bashamohideen, 1992.Regulation of AChE system of fresh water fish, *Cyprinus carpio*, under fenvalerate toxicity, Bull.Environ. Contam.Toxicol.48:18-22.

199. Reddy, P.M., Philip, G.H. (1994) In vivo inhibition of AChE and ATPase activities in the tissues of freshwater fish, *Cyprinus carpio*, exposed to technical grade Cypermethrin. *Bull. Environ. Contam. Toxicol*. 52: 619-626.

200. Remia, K.M, L. Logaswamy, K. Logankumar and D. Rajmohan. 2008.Effect of an insecticide Monocrotophos on some biochemical constituents of the fish*Tilapia mossambica*. Poll.Res.27 (3): 523-525.

201. Rodriguez F G and Gold B G (2000) Environmental monitoring using acetylcholineestrase inhibition *in vitro*. A case study in two Mexican logoons. *Mar. Environ. Res*. 50, 357-360.

202. Roy, S. S. (2002),some toxicological aspects of chlorpyrifos to the intertidal fish, Boleopthalmusdussumieri. Ph.D. Thesis University of Mumbai, India, 52-71.

203. Sachalm, O.W., Jain, N.C. and Carroll, E.J. 1975. Veterinary Hematology, 3rd (ed) Lea and Pebiger, Philadelphia, pp.45.

204. Sahli, T. 1966. In: Text Book of Clinical Pathology (Ed.) Seward, E. Miller, William & Williams & Co., Baltimore, 36.

205. Saleem MA, Shakoori AR (1986). Biochemical effects of sublethal doses of cypermethrin on the sixth instar larvae of *Tribolium castaneum* (Herbst.). Arch. Insect Biochem. Physiol., 3: 447-455.

206. Sampath K, James R and Velammal S (2003) Effect of methyl parathion on blood parameters and its recovery in a catfish, *Mystus kalatius*. *Indian J. Fish*. 50, 191-197.

207. Santhakumar M., , Balaji M., Ramuda K. (1999): Effect of sub lethal concentrations of monocrotophos on erythropoietic activity and certain hematological parameters of fish Anabas testudineus (Bloch). Bull. Environ. Contom. Toxicol .63: 379-384.

208. Sancho E, Cerón J.J. andFerrando, M.D. (2000). Cholinesterase activity and haematological parameters as biomarkers of sublethalmolinate exposure in Anguilla anguilla. Ecotoxicology Environmental Safe, 46: 81–86.

209. Santos, E. A and Nay, L.E.M. (1987): Blood glucose regulation in an estuarine crab Chasmargnathus granulate (Dana, 1851) exposed to different salinities. Comp. Biochem. Physiol., 4: 1033-1035.

210. Satyanarayan S, Bejankiwar R S, Chaudhari P R, Kotangale J P and Satyanarayan A (2004) Impact of some chlorinated pesticides on the haematology of the fish, *Cyprinus carpio* and *Puntius ticto*. *J. Environ. Sci*. 16, 631-634.

211. Satyavardhan, K., 2010. Effect of fenvalerate TM on various tissues of Channa punctatus (Bloch). World Appl. Sci. J., 10: 70-74.

212. Sastry, K. V. and Sharma, K., (1980), Diazinon effect on the activities of brain enzymes from Ophiocephalus (Channa punctatus). Bull. Environ. Contam. Toxicol. Vol (24): 326-332.

213. Sastry, K.V. and Shukla, V 1990. Acute and chronic toxic effects of cadmium on some biochemical and enzymatic parameters in fish. Paper submitted to the second Indian fishries forum held on 27th May, 1990 at Mangalore, Karnataka, India.

214. Sastry, K. V., Samuel, M and Shukla, V., (1993),Metabolic alteration produced by the• organochlorine aldrin in the fresh water teleost fish Channa punctatus. Ind. J. Environ. & Toxicol. Vol (1): 49-56.

215. Saxena K.K., Seth N. (2002). Toxic effects of cypermethrin on certain hematological aspects of fresh water fish, Channa punctatus. Bulletin of Environmental Contamination and Toxicology, 69: 364-369.

216. Schimmel SC, Garnas RL, Patrick JM, Moore JC (1983). Acute toxicity, bioconcentration, and persistence of AC 222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. J. Agric. Food Chem., 31(1): 104-113.

217. Seshigiri Rao, K., Shrinivasa Moorthy, K., Kasraeddy, B., Swami, K.S. and Sreramnly chetty C. (1987) : Effect of benthiocarb on protein

metabolism of fresh water telost, *Sarotherodon mossambicus. Indian. J. Env. Health.* 29: 45-52.

218. Shailendra Kumar Singh, Sunil Kumar Singh and Ram P. Yadav: Toxicological and Biochemical Alterations of Cypermethrin (Synthetic Pyrethroids) against Freshwater Teleost Fish *Colisa fasciatus* at different Season. *World Journal of Zoology*., 2010, 5 (1), 25-32.

219. Shebly EI. A.A. and M.A.H.EI Kady. 2008. Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Tilapia (*Orechromis Niloticus* L.) Res. J. Fisheries and Hydrobiology, 3(2): 84-88.

220. Sheela, M. and Muniadi, S. 1992. Impact of cypermethrin on protein conversion efficiency, RNA, Protein, glycogen content and protease enzyme activity in different tissues of the fish *Lepidocephalichthys thermalis*. Environ. Ecol., 10: 829- 832.

221. Schmidt Nielson B (1975). Osmoregulation: Effect of salinity and heavy metal. Fed. Proc., 33: 2137-2146.

222. Schneider, W.C. (1957) Determination of Nucleic Acids in Tissues by Pentose Analysis. In: Colowick, S.P. and Kaplan, W.O., Eds., Methods in Enzymology, Academic Press, New York, 680-684.

223. Schreck CB, Brouna P (1975) Dissolved oxygen depletion in static bioassay system. Bull Environ Contam Toxicol 14: 149-152.

224. Shoba Rani, A., Sudharshan, R., Reddy, P.V.M and Raju, T.N. (2000): Effect of Sodium arsenate on glucose and glycogen levels in freshwater teleost fish, Tilapia mossambica. Poll. Res., 19(1): 129-131.

225. shodhganga.inflibnet.ac.in/bitstream/10603/69100/9/09_chapter%202.pdf.

226. Shrivastava S, Singh S (2004). Changes in protein content in the muscle of *Heteropneustes fossilis* exposed to carbaryl. J. Ecotoxicol. Environ. Monit., 14: 119-122.

227. Silman I, Sussman JL. Acetylcholinesterase: "classical" and "non-classical" functions and pharmacology. Curr Opin Pharmacol 2005; 5:293-302.

228. Singh, S. P., Chaudhary, R. and Gaur, K. K. 2007. A study of toxicity and behavior of freshwater fish, *Channa punctatus* (Bloch) after intoxication of carbamate and synthetic pyrethroid fenvalerate. *J. Ecophysiol. Occup. Hlth*. 7: 41-45.

229. Singh, K.S., Singh, S.K.S. and Yadav, R.P. 2010.Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost *Colisa fasciatus* at different seasons. World J. Zool., 5(7): 25-32.

230. Sivanatarajan P and Sivaramakishnan T (2013) Studies on some hematologic values of *Oreochromis mossambicus* (Peters) following its sudden transfer to various concentrations ofpotassium chlorate and potassium dichromate. *Eur. J. Appl. Sci*. 5, 19-24.

231. Siva Prasada Rao K, Sambasiva Rao KRS, Sreenivasa Babu K, Ramana Rao KV (1982). Toxic impact of phenthoate on some functional aspects of nitrogen metabolism in the tissue of the fish, *Channa punctatus*. Indian J. Fish., 29: 229-233.

232. Sakr, S.A., S.M. Jamal and AI lail: Fenvalerate induced histopathological and histochemical changes in the Liver of the cat fish Clarias Gariepinus. J. Appl. Sci. Res., 1, 263-267 (2005).

233. Smith TM, Stratton GW (1986) Effects of synthetic pyrethroid insecticides on nontarget organisms. Res Rev 97:93–119.

234. Smith TM, Stratton GW (1986) Effects of synthetic pyrethroid insecticides on non target organisms. Residue Rev 97:93–120 Soderlund DM (2010) State-dependent modification of voltage-gated sodium channels by pyrethroids. Pest Biochem Physiol 97(2):78–86.

235. Sprague, J.B. (1971): The measurement of pollutant toxicity to fish III, sub lethal effect and safe concentration. Water Res., 5: 245-266.

236. Srikanthan, T.N. and Krishnamoorthi, C.R. (1955). Tetrazolium test for dehydrogense. J. Sci. Industrial Res., 1: 206 – 207.

237. Srivastava P and Singh A (2013a) *In vivo* study of effects of dithiocarbamates fungicide (Mancozeb) and its metabolite ethylenethiourea (ETU) on freshwater fish, *Clarius batrachus. J. Biol. Earth Sci.* 3, 228-235.

238. Somogyi, M.J. (1952) Notes on Sugar Determination. Journal of Biological Chemistry, 195, 19-23.

239. Somnuek C, Cheevaporn V, Saengkul C and Beamish F (2007) Variability in acetyl cholinesterase upon exposure to chlropyrifos and carbaryl in hybrid catfish. *Sci. Asia* 33, 301-305.

240. Soreq H, Seidman S. Acetylcholinesterase - new roles foran old actor. Nat Rev Neurosci 2001; 2:294-302.

241. Stamp WG, Lesker PA (1967). Enzyme studies related to sex difference in mice with hereditary muscular dystrophy. Am. J. Physiol., 213: 587.

242. Sprague, J. B, (1969), Water Res.vol-3, pg 793– 821.

243. Sreenivasa MK (1983). Modulation of carbohydrate and associated metabolism in the selected tissues of fresh water mussel *Lamellidens marginalis* during induced methyl parathion stress. Ph.D. thesis, S.V. University, Tirupati, India.

244. Srinivas, R. (1993), Impact of endosulfan on carbohydrate metabolism in the fresh water• fish, Clarias batrachus (Linn.). Ph.D. Thesis, Osmania University, Hyderabad, A.P

245. Stevenson, J.H. 1963. *Royal Soc. Health*, 83(4): pp. 207.

246. Sujay Kumar, G., Reddy, H.M and Reddy, S.M. (2001): Phosphomidon induced changes in the glycolytic potentials of panaeid prawn M. monoceros. J. Acqua. Biol., Vol16(1): 71-76.

247. Swarup, N.,Arora, S.andPathak, S.C. (2002). Laboratory techniques in modern biology. Kalyani publishers, New Delhi-2.

248. Szegletes ,T.,T.Balint ., Z. Szegletes ,and J.Newcsok, 1995.In –vivo effects of Deltamethrine exposure on activity and distribution og molecular forms of carpAChE ,*Ecotoxicol.Environ.Safe* : 31: 258-263.

249. Talas Z S and Gulhan M F (2009) Effects of various propolis concentrations on biochemical and haematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicol. Environ. Saf*. 72, 1994-1998.

250. Tandon S.S., Srivastava P.P., Mukherjee S.C. and Saharan N., 2005. Acute toxicity of synthetic pyrethroids to Indian major carp, Catla catla L., Bull Environ Contam Toxicol, 74:60-613.

251. Taylor, P., 1980. Anticholinesterase agents. In "The Pharmacological basis oftherapeutics" (Ed. A.S Gillman, T.W. Roll and F. Murad (Eds), MacMillan publishing Co., New York; pp 404-466.

252. Thakur, A.C. and Kakde, V.R. 2012. Influence of Cypermethrin on DNA, RNA and RNA/DNA Ratio in Muscles of the Freshwater Fish *Channa punctatus*. Int. Interdisci. Res. Jour., 2: 38-42.

253. The Hindu, daily newspaper, 24th September, 1985.

254. Thenmozhi C, Vignesh V, Thirumurugan R and Arun S (2011) Impacts of malathion on mortality and biochemical changes of freshwater fish, *Labeo rohita*. *Iran. J. Environ. Hlth. Sci. Eng*. 8, 387-394.

255. Thomas Nithyanandam, G., Maruthanayagam, C. and Viswanathan, P. (2007). Effect of sub lethal level of pesticide, Monochrotophos on haematology of *Cyprinus carpio* during the exposure and recovery periods .*Nat. Environ. Poll. Publications*, 6: 615-621.

256. Tilak, K.S., K. Veeraiah and L.V.M. Sastry, A study on the effect of fenvalerate, a synthetic pyrethroid on the carbohydrate metabolism of the Indian bull frog, Rana tigerina (Daud). Asian J. Microbiol. Biotech. Environ. Sci., 3: 369-373 (2001a).

257. Tilak, K.S., K. Veeraiah and S. Vijaya Lakshmi, 2001b. Biochemical changes induced in fresh water fish Labeo rohita (Hamilton) exposed to pesticide mixture. Asian J. Microbiol. Biotech. Environ. Sci., 3: 315-319.

258. Tilak, K.S.,Veeraiah, K and Lakshmi, S.K. (2002): Studies of some biochemical changes in the tissues of Catla catla, Labeo rohita, and Cirrhina mrigala(Hamilton) exposed to NH_3-N,NO_2-N and NO_3-N. J. Env. Biol., 23(4): 377-381.

259. Tilak KS, Satyavardan K, Thathaji PB (2003). Biochemical changes induced by Fenvalerate in the freshwater fish *Channa punctatus*. J. Ecotoxicol. Environ. Monit., 13: 261-270.

260. Tilak. K.S., Wilson Raju, P and Butchiram, M.S. (2009): Effects of alachlor on biochemical parameters of the freshwater fish, Channa punctatus(Bloch). Journal of Environmental Biology., 30(3): 421-426.

261. Tiwari S and Singh A (2004) Toxic and sublethal effects of oleandrin on biochemical parameters of freshwater air-breathing murrel, *Channa punctatus* (Bloch). *Indian J. Exp. Biol*. 42, 413-418.

262. Tiwari S, Ajay Singh (2009). Changes in some biochemical parameters in the liver and muscle of Colisa fasciatus due to toxicity of ethanolic extract of *Nerium indicum* Mill. (Lal Kaner / Latex). Natural Product Radiance, 8(1): 48-54.

263. Tiwari, S., Tiwari, R. and Singh, A. 2012. Impact of Cypermethrin on Fingerlings of Common Edible Carp (*Labeo rohita*). The Scient. World Jour., 1: 1-7.

264. Tripathi, G. (1992). Relative toxicity of Aldrin, Fenvalerate, Captan and Diazon to freshwater for fish *Clarias batranchus*.Biomedical and environmental science 5(1): 33-38.

265. Tripathi, G. and S. Shukla, 1990. Malate and lactate dehydrogenases of a freshwater catfish: Impact of endosulfan. Biomed. Environ. Sci., 3: 52-64.

266. Tripathi, G, ., (2002): J. Environ Bio.vol-23, issue 2, pg 143–146.

267. Tripathi, P. K,., (2003), Asian fisheries Sci. issue 16, pg 349–359.

268. Tripathi P K and Singh A (2003) Toxic effects of dimethoate and carbaryl pesticides on protein metabolism of the freshwater snail *Lymnaea acuminata*. *Bull. Environ*. *Contam. Toxicol*. 70,146-152.

269. Tripathi, G. and Verma, P. 2004. Fenvalerate induced changes in a catfish, Clarias batrachus: metabolic enzymes, RNA and protein. Comp. Biochem. Physiol., 138C: 75-79.

270. Torre F R, Ferrari L and Salibian A (2002) Freshwater pollution biomarker: response of brain acetylcholinesterase activity in two fish species. *Comp. Biochem. Physiol*. 131C, 271-280.

271. Ullah R, Zuberi A, Ullah S, Ullah I and Dawar F U (2014) Cypermethrin induced behavioural and biochemical changes in mahseer, *Tor putitora*. *J. Toxicol. Sci*. 39, 829-836.

272. Umminger, B.L., 1970. Physiological studies on *Fundulus beterolitus* III. Carbohydrate metabolism and survival of sub-zero temperature. J.Exp.Zool. 170: 76- 81.

273. Umminger, B.L. (1977): Relation of whole blood sugar concentrations in vertebrates to standard metabolic rate. Comp. Biochem. Physiol., 56A: 457-460.

274. US Environmental Protection Agency (1987) Pesticide Fact Sheet Number 164: Cyfluthrin. U.S. EPA, Office of Pesticide Programs. Washington, DC.

275. USEPA, 1992 Pesticide environmental fate one line summary; cyfluthrin. Environmental Fate and Effects Division. Environmental Protection Agency, Washington DC, USA, 1992.

276. Van Loon, A.A.W.M., Groenendijk, R.H., Van DerShcanslohman, P.H.M. and Bran, R.A. 1991. Detectionof induced damage in DNA in human blood exposed to ionic radiation at biologically relevant doses. Int. J. Rad. Biol., 59: 651-660.

277. Vasantharaja, C., Pugazhendy, C., Meenaambai, M., Prabukaran, S. and Dar, N.A. 2013. Properties of *Cardiospermum halicacabum* against the toxic effect of cypermethrin in the quantification of nucleic acids in the freshwater fish *Cirrhinus mrigala* (Hamilton). Int. Res.J. Pharm., 4(1): 280-283.

278. Veeraiah, K. and M.K. Durga Prasad: Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish. Labeo rohita (Hamilton). Proc. Acad. Environ. Biol., 7, 143-148(1998).

279. Veeraiah, K., Vivek, Ch., Srinivas Rao, P. and Venkatrao, G. 2013. Biochemical Changes induced by Cypermethrin (10% EC), A Pyrethroid Compound in Sublethal and Lethal Concentrations to the Freshwater Fish *Cirrhinus mrigala* (Hamilton). J. Atom. Mol., 3(6): 625-634.

280. Veeraiah, K.. 2001. Cypermethrin Toxicity and its impact on histochemical and Histological changes in the Indian major carp *Labeo rohita* (Hamilton). Ph.D thesis, Nagarjuna University, Guntur, India.

281. Venkataramana, G.V., P.N. Sandhya Rani and 14. P.S. Murthy, 2006. Impact of Malathion on the biochemical parameters of gobiid fish, Glossogobius giuris (Ham). J. Environ. Biol., 27: 119-122.

282. Verma, S.R., Rani, S., Bansal, S.K. and Delala, R.C. 1980. Effect of pesticide thiotox, dichlorovous and chlorofurun on the fish Mystus vittatus. Water, Air and Soil Pollution, 13(2): 229-234.

283. Vrhovae, G. V and D. Zeljezic, "Evaluation of DNA damage in workers occupationally exposed to pesticide using SCG assay. Pesticides genotoxicity revealed by comet assay", Mut. Res., 469, 279-285, 2000.

284. Vijverberg HPM, Van den Bercken J. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit Rev Toxicol 1990; 21:105-126.

285. Visweswaraiah, K.M., Jayaram, and S.K. Majumdar, 1975. Environmental pollution with pesticides. *J. Food. Sci. Tech., 2 :* 53-60.

286. Visweswaraiah and Jayaram, 1972. As cited by N.V. Nanda Kumar, S.V.University, Tirupati. On environmental monitoring of pesticides residues in Some districts of Andhra Pradesh.

287. Vishwaranjan, S., S. Beena and A. Palavesam, 1988. Effect of tannic acid on Protein, Carbohydrate and Lipid level in the tissue of the fish *Oreochromis mossambicus*. Environ. Ecol., 6: 289-292.

288. Visvanathan, P., Maruthannayagam, C and Govinda Raju, M. (2009): Effect of malathion and endosulfan on biochemical changes in Channa punctatus. J. Ecotoxicol. Environ. Monit., 19(3): 251-257.

289. Walton D G, Acton A B and Stich H F (1984) DNA repair synthesis following exposure to chemical mutagens in primary liver, stomach and intestinal cells isolated from rainbow trout. *Cancer Res*. 44, 1120-1121.

290. Wang C L, Guis J C and Wang P (2009) Sublethal effects of pesticides mixtures on selected biomarkers of *Carassius auratus. Environ. Toxicol. Pharmacol*. 128, 414-419.

291. Waugh, A., Grant, A. W., and Ross, J. S. (2001). Ross and Wilson Anatomy and Physiology in Health and Illness. 9 th edition, Churchill Livingston, an imprint of Elsevier Science Limited. 59-71.

292. Well, R., Baldwin, J., Seymour, R., Chritian, K. and Brittain, T. (2005).Red blood cell function and haematology in two tropical freshwater fishes from Australia; *Comp. Biochem, Physiol.* A 141: 87-93.

293. Wolansky, M.J., Harrill, J.A., 2008. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. Neurotoxicol. Teratol. 30, 55–78.

294. World health organization (WHO) (1986) Environmental health criteria no.63. Organophosphorous insecticides a general introduction, World Health Organisation, Geneva, p-181.

295. Worthing, C.E. (ed.) (1991) The Pesticide Manual: A World Compendium. Ninth edition. The British Crop Protection Council.

296. Yaji, A.G., J. Auja, S.J. Oniye, J.A. Adakole and J.I. Usman, 2011. Effect of Cypermethrin on behavior and biochemical indices of freshwater fish, Oreochromis niloticus.

297. Yekeen TA, Fawole OO. Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*. Afr J Biotechnol. 2011;10:14090–14096. doi: 10.5897/AJB10.2468.

298. Younos TM, Weigmann DL (1988) Pesticides in groundwater data base. 1988 Interim Rpt. U.S. Environmental Protection Agency. Washington D.C.

299. Young, S. J., Dowman, A and Cowell, D. C.: The detection of pentachlorphenol by its inhibitory effectiveness on lactate dehydrogenase of rabbit muscle and bovine heart. *Pestic. Biochem. Physiology.*, 1999,64, 1-8.

300. Yousef MI, Awad TI, Mohamed EH. Deltamethrin induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology 2006; 227(3):240-247.

301. Zitko, V., McIntrye, A.D and Mills, C.F. (1975): Potential persistent industrial organic chemicals other than PCB. In: Ecological Toxicology Research. Plenum Perss. New York.197.

.

LIST OF PUBLICATIONS

International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.6, Issue.3, pp.20-30, June (2019) **E-ISSN:** 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v6i3.2030

Effects of synthetic pyrethroids Cyfluthrin and Fenvalerate on Nucleic acid contents of fresh water fish *Gambusia affinis*

S. Mohammed Ghouse

Department of Zoology, Osmania College, Kurnool, Andhra Pradesh, India

Corresponding Author: syed0002001@gmail.com, Mobile: 9501603265

Available online at: www.isroset.org

Received: 22/May/2019, Accepted: 12/Jun/2019, Online: 30/Jun/2019

***Abstract*-**India is a country with more than 60–70% of its population dependent on agriculture. Rapid industrialization and successful green, white and blue revolutions have necessitated a large variety of chemicals/pesticides usage resulting environmental pollution which has become not only a national but a global problem. Fish occupy a prominent position in the field of aquatic toxicology and have been employed in studies concerning ecological health. In the present investigation two synthetic pyrethroids were selected namely Cyfluthrin and Fenvalerate to evaluate their toxicity on *Gambusia affinis.* In the present study there is significant decline in DNA content in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. Decreases in the DNA content due to inhibition of the enzymes in DNA synthesis. The declines in RNA level in treated fish due to obstruction in RNA synthesis. Resulting in the swelling and chromatolysis of Nissle bodies which are rich in RNA. Pesticides may influence directly or modify DNA, other cellular process associated with the integrity of the genome.

Keywords : India, agro-based country, pesticides, fish, DNA, RNA decline

I. INTRODUCTION

India is primarily an agro-based country with more than 60–70% of its population dependent on agriculture. Rapid industrialization and successful green, white and blue revolutions have necessitated a large variety of chemicals usage resulting environmental pollution which has become not only a national but a global problem. More than 30% of its agricultural productivity is lost due to the to pests .Excess use of insecticides to get more agriculture yield and spraying of pesticides improperly in agricultural fields cause these chemicals to accumulate in the field, along with rain water these pesticides finally reach the natural water systems like ponds, streams, lakes and rivers., it will ultimately lead to alteration in the ecosystem . [1].. Pesticides are hazardous to the biodiversity including the man. The synthetic pyrethroids act as excitatory nerve poisons, thought to poison by interfering with the ionic permeability of nerve cell membranes. Through a better understanding of toxicology and toxicity testing in relation to these biochemical parameters in non-target organisms like fish we may be able to contribute to such an inquiry and hence the present work**.**

Various pesticides that are commonly used in pest control programmes are generally grouped under 4 heads: They are:
(1) chlorinated hydrocarbon, the 1st generation insecticides (aldrin, DDT, chlordane, dicofol (kelthane), dieldrin, endrin, endosulphon, heptachlor, kepone, lindane, methoxychlor, mirex, and toxaphene];
(2) the second generation organophosphate insecticides include, parathion, malathion, diazinon, monocrotophos etc.,
(3) the third generation insecticides are exemplified by carbamates like, carbaryl, carbofuran, propuxur etc., and
(4) the fourth generation insecticides are the pyrethroids example , Allethrin

Fish occupy a prominent position in the field of aquatic toxicology and have been employed in studies concerning ecological health. After China, India is the second largest producer of pesticides in Asia and with 12th ranks globally. India started the production of BHC in 1952 with the establishment of a plant near Calcutta. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat. In India the usage pattern of the of pesticide is different from the world in general. Globally 44% of the pesticide is used as inscecticide , where as in india 76% of the pesticide used as insecticide . [2]. The potential toxicity of the pesticide will lead to tissue damage and change in behaviour . [3,4].

© 2019, IJSRBS All Rights Reserved

The detoxification mechanism present in the body of the organism will work to get rid of foreign substances. If the concentration of toxic substance is higher it results in damage at cellular or even at molecular level, which leads to behavioral, physiological, pathological and biochemical disorders that may prove fatal conditions to aquatic life [5,6,7,8]. The protection of the genetic diversity in natural populations maintained through DNA integrity [9]. DNA and RNA damage , health assessment of organism can be done by detection of structural/functional disturbances in the DNA [10]. The main purpose of this study is to evaluate the toxicity of cyfluthrin and fenvalerate on , *Gambusia affinis* with reference nucleic acid content.

II. MATERIALS AND METHODS

Collection, maintenance and acclimatization of experimental fish

Adult fresh water fishes *Gambusia affinis* with weight (0.7-1.5 gm), length (2.3 - 3.2 cm) from both sexes were selected randomly for the experimental studies were collected from water bodies and fisheries department of Kurnool district , Andhra Pradesh , India. The collected fish were transported in polythene bags filled with half water with least disturbance . They are kept in aquarium tanks containing well aerated de-chlorinated water to acclimatize to the laboratory conditions, with physico-chemical characteristics : temperature 24±2°C, pH 7.1±0.2 at 24°C, dissolved oxygen 9.6±0.8 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as $CaCO_3$/L, phosphate 0.39±0.002 µg/L, salinity in different cement tanks for one week . To prevent fungal infection the fish tanks were washed with potassium permanganate solution. Dead fish were removed as soon as possible to avoid water fouling.

Fishes were fed daily on commercial fish food. The unused food is removed daily and water was renewed daily .Test fishes were screened critically for any signs of stress, disease, physical damage and mortality. Injured, severely, diseased fishes were discarded. The fishes were exposed to sublethal concentrations for treated and control period of 96 hours. A control group was maintained with identical environment. The toxicant water as well as normal water is renewed every day. The fish were sacrificed from both experimental and control groups on 96 hours.

Selection of pesticides

In the present investigation two synthetic pyrethroids were selected namely Cyfluthrin and Fenvalerate.

Cyfluthrin

As per the reports pyrethroids are extremely toxic to fish and to some beneficial aquatic arthropods like lobster and shrimp. Cyfluthrin first registered by EPA in 1987, cyfluthrin is found in both restricted use (RUP) and general use insecticides [11]. Cyfluthrin is clear liquid, dark amber in color, with an oily to pasty consistency, and has a faint aromatic solvent odor at room temperature. Cyfluthrin, cyano (4-fluoro-3- phenoxyphenyl) methyl-3-(2,2 dichloroethenyl) -2,2-dimethylcyclopropanecarboxylate) is a broad spectrum synthetic type II pyrethroid insecticide.

Cyfluthrin acts as contact and stomach poison it attacks the nervous system; a nonsystemic insecticide used to control chewing and sucking insects and also in public health situations [12]. Insects when in come in contact with cyfluthrin die of starvation and desiccation, ceasing to feed. It is extensively used in agricultural crops, stored products, public health situations (i.e. cockroaches, mosquitoes, and flies), ornamentals, turf, and domestic pests. Insects like cockroaches, ants, grain beetles, silverfish, , fleas, flies, European corn borer, Colorado potato beetle, and many others are the main target of cyfluthrin use. [13,14]..

Cyfluthrin act on non target organisms. The toxicity of cyfluthrin observed in various fish species is 0.68mg/L inrainbow trout (*Oncorhyncus mykiss*), 1.5mg/L in bluegill (*Lepomis macrochirus*), 22mg/L in carp (Cyprinus carpio), 3.2mg/L in golden orfe (*Leuciscus idus*), channel catfish (*Ictalurus punctatus*) and sheepshead minnow (*Cyprinodon variegatus*), 25.82mg/L for 48h [15]. and 21.07mg/L for 72 h to Nile tilapia fry (*Oreochromis niloticus* L. 1758) [16]. Cyfluthrin accumulation in the flesh of *Tilapia nilotica*, exposed to a sublethal dose of 0.001 mg/L, was 0.009 mg/L after 5 days of application [17]. also pyrethroid toxicology in mammals, birds, amphibians and both terrestrial and aquatic invertebrates.reviewed. [15,18].

Cyfluthrin causes oxidative damage in aquatic organisms resulting in the damage of essential cell components like DNA, protein, lipids and lipoproteins, caused by free oxygen radicals [19]. Thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes [20]. The products which has cyfluthrin are classified as acute Toxicity Category II (bearing the signal word "Warning") or Toxicity Category I (bearing the signal word "Danger") by EPA based on its potential ability to cause eye damage [21].

© 2019, IJSRBS All Rights Reserved

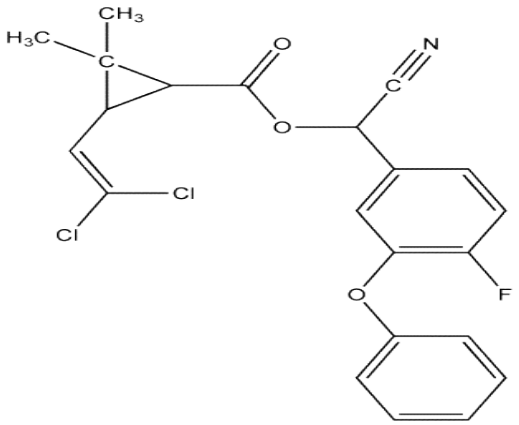


Figure 1. Cyfluthrin cyano (4-fluoro-3-phenoxyphenyl)methyl-3-(2,2- dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate

Fenvalerate

Fenvalerate, is a clear viscious yellow liquid with a mild odor, a synthetic pyrethroid. First developed in 1974 by Sumitomo Chemical Co. of Japan (Sumicidin™), is most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. The biology of the non target species is affected by the use of insecticide along with pests. [22,23,24,25,26,27,28]. In India, the pesticide is used primarily to control pests of cotton and vegetables [29].

The LC_{50} for 48 h of fenvalerate exposure to carp, *C. carpio* (8 cm) at p H 8-8.88 was reported to be 0.0210 mgL-1 [30]. Acute toxicity (LC_{50}) calculated over 96h for catla fingerlings (2.02 ± 0.1 g) was observed to be 6 µgL-1 [31]. $_{LC50}$ value of fenvalerate at 96 hours to *Channa punctatus* (20 ± 3 g) is reported to be 2.13 µg L-1 [32]. Fish have a poor ability to metabolise and excrete fenvalerate [33]. and thus are susceptible to even minute concentration of the pesticide.

Fenvalerate have half life of approximately two to four weeks in mineral and organic soils [34]. Deltamethrin is more persistent with a half-life of more than two months in soils [35]. . Fenvalerate is recently developed synthetic pyrethroids, which is highly toxic to fish but shows very low avian and mammalian toxicity. [36,37].

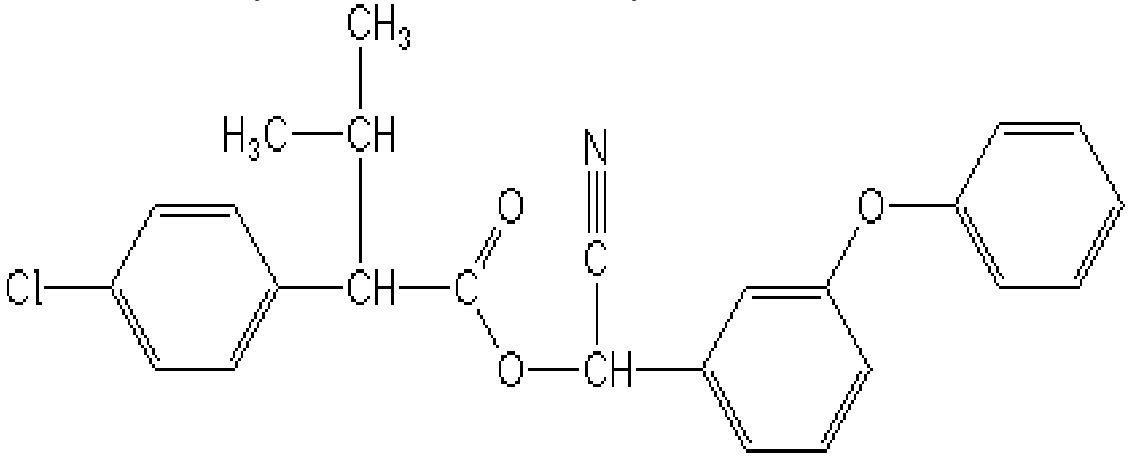


Figure 2. IUPAC name : (RS)-a-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate
Chemical Abstracts name : cyano (3-phenoxyphenyl)methyl 4-chloro-a-(1-methylethyl) benzene acetate

Experimental design

Group 1 : Fish exposed to tap water observed for 24 to 96 Hours (Untreated control).

Group 2 : Fish exposed to sublethal concentration of cyfluthrin of 24 to 96 hours.

Group 3 : Fish exposed to sublethal concentration of fenvalerate of 24 to 96 hours.

© 2019, IJSRBS All Rights Reserved

Figure 3. ***Gambusia affinis***

Calculation of LC $_{50}$

The LC $_{50}$ values were obtained by probit analysis method based percentage of test animals surviving at concentrations that were lethal to more than half and less than half of the test subjects [38].

Acute toxicity studies : The toxicity studies will conducted by using the technical grade formulation available formulation of Fenvalerate and Cyfluthrin. Solutions of desired concentrations were prepared in 95% acetone to get the stock solution as well as working solution as 100mg/100ml and 1mg/1ml of toxicant chemical Fenvalarate and Cyfluthrin in both technical formulation use of acetone in control as recommended by EPA will be followed**.**

The the acclimatized fishes were kept in cement tanks and ten fishes in two groups control fishes will be kept in dechlorinated water only. Each set of experiment was replicated six times. Mortality was recorded every 24h during the observation period of 96h.

The LC $_{50}$ values were calculated for *Gambusia affinis* exposed to of cyfluthrin and fenvalerate at 3, 6,12,24, 48, 72 and 96 hours and they were found to be 36.12, 34.41, 33.76, 31.32, 28.97, 26.69, 24.73 **µg/L –** cyfluthrin and 19.67, 18.98, 17.29, 16.98, 15.93, 14.75, 13.87 **µg/L -** fenvalerate respectively .The toxicity curve showed the mode of action and the toxicity of cyfluthrin and fenvalerate for different concentrations at different periods. A similar result with regard to fenvalerte toxicity in *Gambussia affinis* , the LC $_{50}$ value of 15.0 **µg** /L (48 h) was reported . [39].

In the present study, it was observed that the mortality rate increased with the higher concentration of cyfluthrin and fenvalerate over a short period of time and diminished with decreasing concentration during the subsequent hours of treatment.

Statistical Analysis

The data obtained from the quantitative study were expressed as the mean ± S.E. The mean values were calculated from 6 individual observations. P—values were calculated by the students 't' test.The two mean values obtained were considered significant from each other with standard error.

Estimation of nucleic acids

Nucleic acids were extracted from the tissues by the method described by Schneider (1957). [40].

Principle: Nucleic acids are separated from the other tissue components by exploiting their property of preferential solubility in hot trichloroacetic acid (TCA) solution.

Procedure: Known weight of tissue was rinsed finely and homogenized in distilled water, to homogenate 5 ml of 10% ice cold TCA was added and was kept in an ice bath for 30 minutes to allow complete precipitation of proteins and nucleic acids. The mixture was centrifuged and the precipitate was later treated with absolute alcohol and centrifuged to remove lipid materials. The precipitate free of lipids was suspended in required amount of 5% TCA and placed in a water bath maintained at 90°C for 15 minutes with occasional stirring which facilitates the quantitative separation of nucleic acids from the precipitated proteins. This was centrifuged and the supernatant was used for the estimation of Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA). [41].

© 2019, IJSRBS All Rights Reserved

Estimation of DNA

Principle: Estimation of DNA was performed by using diphenylamine reagent (Schneider, 1957). This method is based on the property of deoxyribose moiety of DNA to form hydroxy laevulaldehyde in TCA solution. This reacts with diphenylamine to give a coloured complex. The intensity of colouration is presumed to be proportional to the pentose (deoxyribose) concentration in the DNA hydrolyzate. Total DNA content was expressed as mg/100g wet tissue.

Reagents A. Dische's diphenylamine reagent 1 g of diphenylamine was dissolved in 100 ml of glacial acetic acid. After adding 2.75 ml of Conc. H2SO4, the solution was mixed and stored in dark. B. Standard DNA Solution Freshly prepared by dissolving 100 mg of commercially available DNA with 100 ml of distilled water.

Procedure: 1ml of isolated nucleic acid TCA hydrolyzate was taken and mixed well with 0.5 ml of perchloric acid. The solution was kept for boiling after adding 2 ml of reagent A. Boiling was done in water bath for 10 minutes. The solution was then cooled under tap water and optical density was measured at 565 nm against blank. [41].

2.12.2. Estimation of RNA

Principle : Total RNA content was estimated by the methods of Schneider (1957) using orcinol reagent. The ribose moiety of RNA in the TCA extract is converted to furfural which condenses with orcinol to yield a blue coloured complex. The colour intensity is presumed to be proportional to RNA concentration in the extract. Total RNA content was expressed as mg/100g wet tissue.

Reagents A. Orcinol reagent 500 ml of 30 % HCl was taken and 1 g of orcinol was dissolved well in it. 4-5 ml of 10 % ferric chloride was added and mixed in it. The solution was stored in a brown bottle. B. Standard RNA Solution dissolved 100 mg of RNA in 100 ml of distilled water

Procedure: 2 ml of nucleic acid TCA extract was taken and added with 2 ml of orcinol reagent. The solution was mixed and kept for boiling for 20 minutes and cooled thereafter. Absorbance was read at 670 nm in UV- spectrophotometer. [41].

III. RESULTS

Deoxyribonucleic acid (DNA)

Gill

The content of DNA in gill tissues are decreased significantly in the present study when *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control with cyfluthrin (group-2) are - 16.73, - 22.22, - 27.08, - 32.35 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in DNA content over the control are -20.21, 30.05, - 39.19, - 43.94 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1: Fig.4)..

Liver

The observed value of DNA content in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percent decline in DNA content with cyfluthrin (group-2) are- 19.48, - 27.88, - 30.37, - 38.63 for 24, 48, 72 and 96 hours respectively. The percent decrease in DNA content with fenvalerate (group-3) are - 23.84, - 32.38, - 45.33 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels ((Table 1: Fig.4).

Kidney

The DNA content of the in the tissues of kidney in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are -9.92, - 16.83, - 19.62, - 23.90 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .

The percentage of alterations in fish exposed to fenvalerate (group-3) are - 11.26, - 19.57, - 28.07, - 34.39 for 24, 48, 72 and 96 hours respectively. The levels of DNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1: Fig.4).

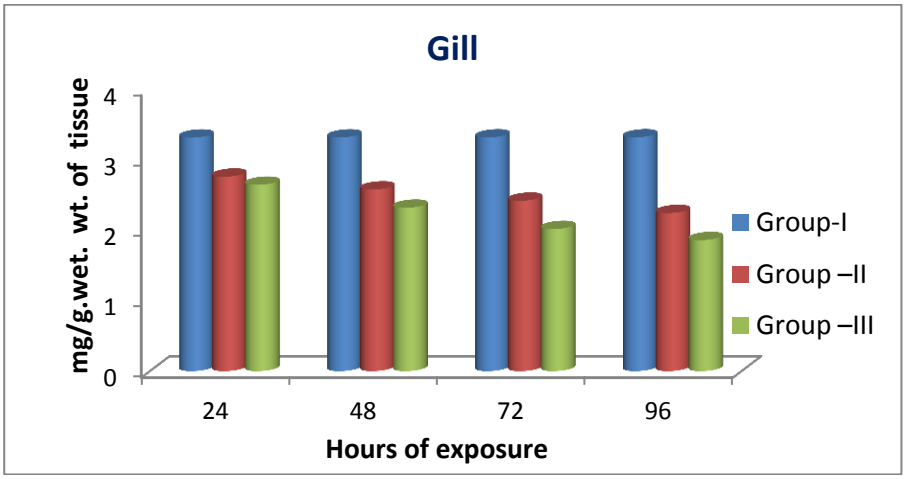
© 2019, IJSRBS All Rights Reserved

Table 1 : Variations of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure			
		24	48	72	96
Gill	Group-I Control	3.310 ± 0.045	3.311 ± 0.043	3.312 ± 0.042	3.313 ± 0.040
	Group –II- Cyfluthrin % COC	2.756* ± 0.065 % - 16.73	2.575** ± 0.064 % - 22.22	2.415** ± 0.065 % - 27.08	2.241** ± 0.066 % - 32.35
	Group –III- Fenvalerate % COC	2.641* ± 0.065 % -20.21	2.316** ± 0.064 % -30.05	2.014** ± 0.065 % - 39.19	1.857** ± 0.066 % - 43.94
Liver	Group-I Control	5.721± 0.674	5.722± 0.672	5.722± 0.671	5.723± 0.669
	Group –II- Cyfluthrin % COC	4.606* ± 0.733 % - 19.48	4.129** ± 0.737 % - 27.88	3.984** ± 0.739 % - 30.37	3.512** ± 0.740 % - 38.63
	Group –III- Fenvalerate % COC	4.357* ± 0.733 % - 23.84	3.869** ± 0.737 % - 32.38	3.128** ± 0.739 % - 45.33	2.567** ± 0.740 % -55.14
Kidney	Group-I Control	4.412 ± 0.049	4.413 ± 0.047	4.413 ± 0.046	4.414 ± 0.048
	Group –II- Cyfluthrin % COC	3.974* ± 0.670 % -9.92	3.670* ± 0.672 % - 16.83	3.547** ± 0.674 % - 19.62	3.359** ± 0.680 % - 23.90
	Group –III- Fenvalerate % COC	3.915* ± 0.542 % - 11.26	3.549* ± 0.546 % - 19.57	3.174* ± 0.550 % - 28.07	2.896* ± 0.553 % - 34.39

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels (+,-) denotes decreased and increased .%COC (change over control) ;

Fig. 4 : Changes in the content of Deoxyribonucleic acid (DNA) (mg/g wet. wt of tissue) in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours



© 2019, IJSRBS All Rights Reserved

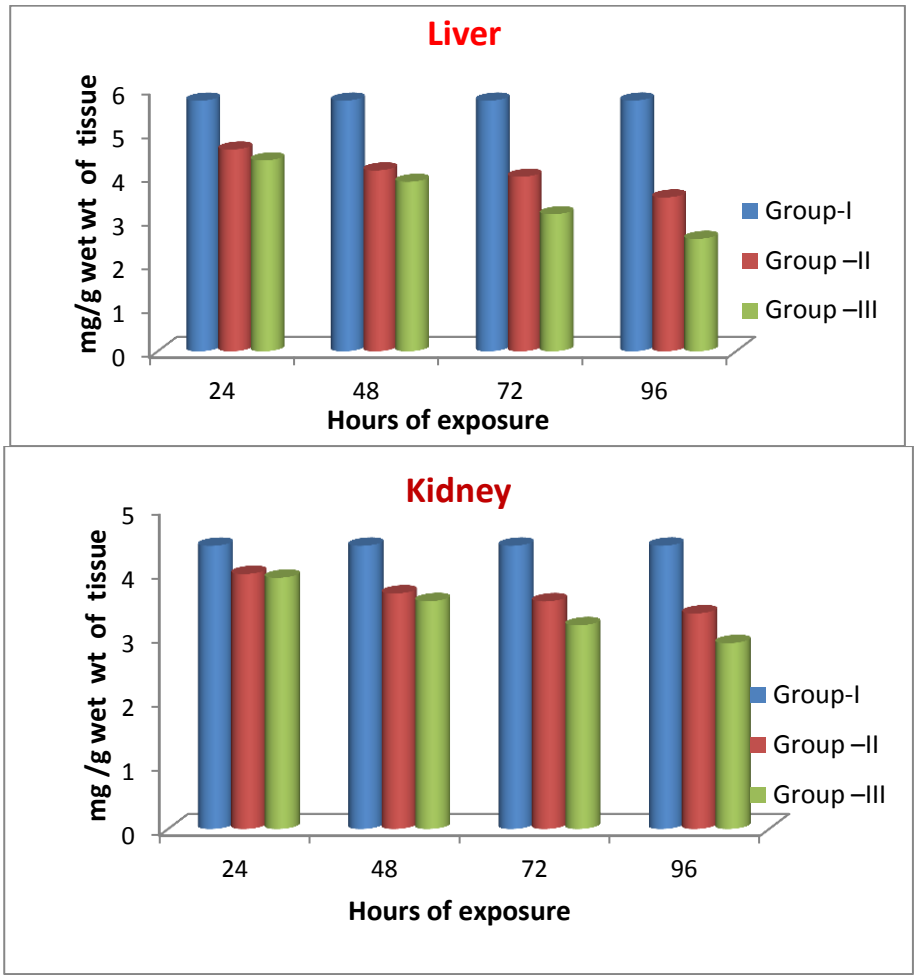


Table 2 : Variations of Ribonucleic acid (RNA) (mg/g wet. wt of tissue) content in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure			
		24	48	72	96
Gill	Group-I Control	2.43± 0.121	2.43± 0.122	2.44± 0.122	2.44± 0.123
	Group –II- Cyfluthrin % COC	2.31* ± 0.145 % - 4.93	2.28* ± 0.146 % - 6.17	2.18** ± 0.145 % - 10.65	2.01** ± 0.145 % - 17.066
	Group –III- Fenvalerate % COC	2.28* ± 0.453 % - 6.17	2.21* ± 0.456 % - 9.05	2.09** ± 0.458 % - 14.34	1.96** ± 0.457 % - 19.6
Liver	Group-I Control	4.98 ± 0.221	4.99 ± 0.222	4.98 ± 0.224	4.99 ± 0.223
	Group –II- Cyfluthrin % COC	4.81* ± 0.323 % - 3.41	4.67* ± 0.324 % - 6.41	4.49** ± 0.324 % - 9.83	4.38** ± 0.326 % - 12.24
	Group –III- Fenvalerate % COC	4.75* ± 0.452 % - 4.61	4.53* ± 0.457 % - 9.03	4.37** ± 0.458 % - 12.24	4.21** ± 0.459 % - 15.63
	Group-I Control	3.10 ± 0.115	3.11 ± 0.114	3.11 ± 0.115	3.12 ± 0.116

© 2019, IJSRBS All Rights Reserved

Kidney	Group –II- Cyfluthrin % COC	3.01*± 0.145 % - 2.90	2.93* ± 0.147 % - 5.78	2.84 **± 0.148 % - 8.68	2.74** ± 0.149 % - 12.17
	Group –III- Fenvalerate % COC	2.98*± 0.261 % - 4.18	2.90* ± 0.263 % - 6.75	2.81 **± 0.267 % - 9.64	2.69** ± 0.298 % - 13.78

Values are mean ± S.E Mean of six individual observations and student t-test. significant at * P<0.05; Significant at * P<0.01 levels(+,-) denotes decreased and increased .%COC (change over control) ;

Ribonucleic acid (RNA)

Gill

In the present study RNA content in the tissues of gill are decreased in the present study *Gambusia affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) , when compared with the control group. The percentage of decrease over the control with cyfluthrin (group-2) are - 4.93, - 6.17, -10.65, - 17.066, for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decreased in RNA content over the control are - 6.17, - 9.05, - 14.34, - 19.6 for 24, 48, 72 and 96 hours respectively. The levels of RNA content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 2: Fig.5).

Liver

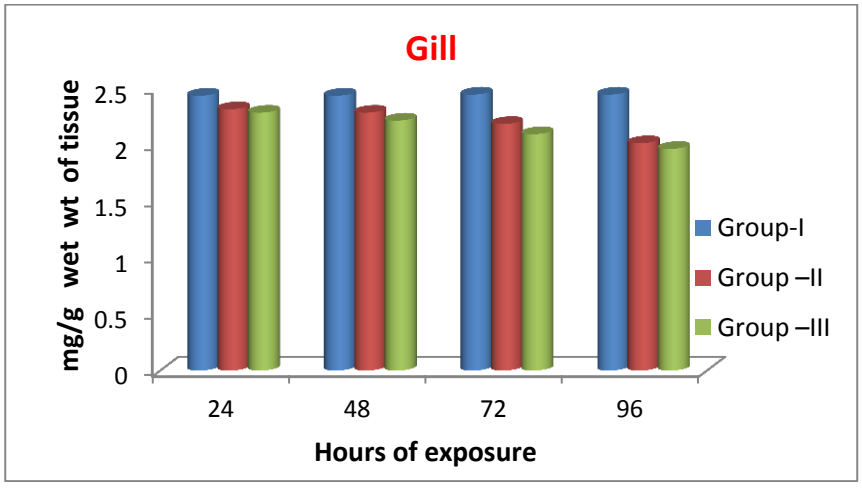
The observed value of RNA content in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a decline , when compare with the control group. The percent decline in RNA content with cyfluthrin (group-2) are - 3.41, - 6.41, - 9.83, - 12.24 for 24, 48, 72 and 96 hours respectively. The percent decrease in RNA content with fenvalerate (group-3) are - 4.61, - 9.03, - 12.24, - 15.63 for 24, 48, 72 and 96 hours respectively. The levels of RNA content content for 3 groups are recorded are statistically significant at 1% and 5% levels(Table 2: Fig.5).

Kidney

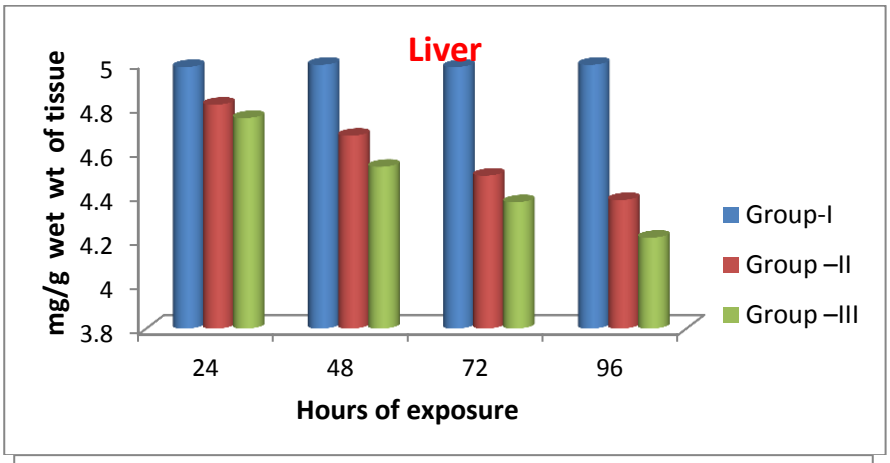
In the present investigation the RNA content of the in the tissues of kidney in *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 2.90, - 5.78, - 8.68, - 12.17 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2).

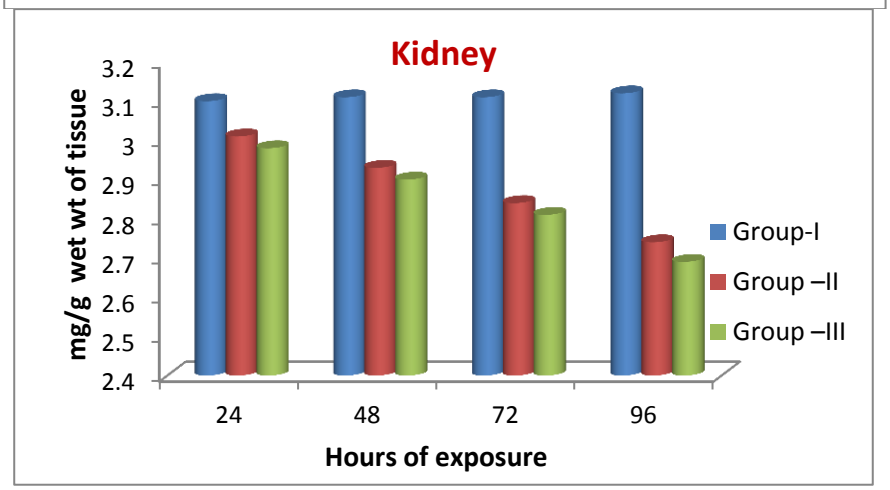
The percentage of alterations in fish exposed to fenvalerate (group-3) are - 4.18, - 6.75, - 9.64, - 13.78 for 24, 48, 72 and 96 hours respectively. The levels of RNA content content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 2: Fig.5).

Fig. 5 : Changes in the content of Ribonucleic acid (RNA) (mg/g wet. wt of tissue) in fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours



© 2019, IJSRBS All Rights Reserved





IV. DISCUSSION

In the present study there is significant decline in DNA content in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate . Pesticides appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. In fish, DNA repairing at a much lower speed than in mammals [42,43]. The toxicant decreases the DNA content due to inhibition of the enzymes in DNA synthesis . It is known that DNA functions as a primer in DNA and RNA polymerase reactions [44]. , and the inhibition in DNA content can result in the inhibition of both DNA and RNA synthesis. Alteration in nucleic acid content leads to variations in protein profile [45,46]. The process of transcription control the activities of all the enzymes , when the transcription process is curtailed, mRNA and protein synthesis does not occur, due to which metabolism is impaired.

In the present study there is significant decline in RNA content in the gill, liver and kidney tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The production of RNA plays a vital role in protein synthesis. The inhibition of RNA synthesis at transcription level may affect the protein content [47]. Significant decrease of RNA observed in the presen study . Pesticides may influence directly or modify DNA, other cellular process associated with the integrity of the genome. The pesticides interacts with the cellular DNA produces a variety of primary lesions such as single strand breaks, double strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases [48].

Similar results were also reported by various investigations: (*Cyprinus carpio*); [49]. (*Cirrhinus mrigala*); [50]. (*Channa punctatus*); [51]. (*Labeo rohita*); [52]. (*Colisa lalia*); [53]. (*Cirrhinus mrigala*); [54]. (*Channa striatus*); [55]. (*Channa punctatus*); [56]. which substantiate the results of this study suggesting that cypermethrin is a potent inhibitor of nucleic acid synthesis even at sub lethal concentrations

Pesticides are electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway. Protein level is depleted in tissues of fish because RNA plays significant role in protein synthesis [57]. The decrease in RNA level will affect the protein synthesis. [58]. reported similar results. Under stressed conditions animals

© 2019, IJSRBS All Rights Reserved

require more energy to overcome the effect of pescticide. As a result, glucogenesis increases in comparsion with protein synthesis. The significant declines in RNA level in treated fish due to obstruction in RNA synthesis. Nissle bodies are rich in RNA, the swelling and chromatolysis of Nissle bodies is caused by pesticides. [59]. Decrease in protein synthesis, impairment of nucleic acid metabolism, the degradation of cells upon exposure to pesticides may be due to, resulting in the reduction in the DNA and RNA content.

V. CONCLUSION

It can be concluded that there is correlation between fish cyfluthrin and fenvalerate exposure and alterations in the nucleic acid contents in selected tissues. The DNA and RNA contents in tissues of gill, liver and kidney are gradually decreased in experimental fish in comparison with the control group. Decreases in the DNA content due to inhibition of the enzymes in DNA synthesis. The declines in RNA level in treated fish due to obstruction in RNA synthesis, resulting in the swelling and chromatolysis of Nissle bodies which are rich in RNA.

REFERENCES

[1]. Odum, E.P., 1971. Fundamentals of Ecology (W.B.Saunders), Toppanlo, Ltd., Tokyo, Japan.
[2]. Mathur , S.C. 1999. Future of Indian pesticides industry in next millennium. Pesticide Information. 24(4): 9-23.
[3]. Latner, A.L., 1975. In: Clinical biochemistry (Ed.: Contarow and Trumper). Saunders Company, Philadelphia, pp: 182-192.
[4]. Venkataramana, G.V., P.N. Sandhya Rani and 14. P.S. Murthy, 2006. Impact of Malathion on the biochemical parameters of gobiid fish, Glossogobius giuris (Ham). J. Environ. Biol., 27: 119-122.
[5]. Anitha Susan, T.1999. Toxicity and effect of synthetic pyrethroid fenvalerate on the metabolism of the three Indian major carps *Labeo rohita*, *Catla Catla* and *Cirrhinus mrigala* (Ham). Ph.D. Thesis submitted to Nagarjuna University, A.P. India.
[6]. Rathod, D.S., M.V. Lokhande and V.S. Shembekar, 2009. Toxic impact of the biochemical composition of vital tissues of fish, Arias dussumieri. Shodh, Samiksha and Mulyankan, 2: 147-149.
[7]. Satyavardhan, K., 2010. Effect of fenvalerate TM on various tissues of Channa punctatus (Bloch). World Appl. Sci. J., 10: 70-74.
[8]. Yaji, A.G., J. Auja, S.J. Oniye, J.A. Adakole and J.I. Usman, 2011. Effect of Cypermethrin on behavior and biochemical indices of freshwater fish, Oreochromis niloticus.
[9]. Farah MA, Atteq B, Ali MN and Ahmad W. Evaluation of genotoxicity of PCP and 2,4d by micronucleus test in freshwater fish, *Channa punctatus*. *Ecotoxicol. Environ. Saf.* (2003)54: 25-29.
[10]. Handy RD, Jha AN and Depledge MH. Biomarker approaches for ecotoxicological biomonitoring at different levels of biological organization. In Burden, F., McKelview, I., Forstner, U., Guenther, A (eds). *Handbook of Environmental Monitoring*, MCGraw Hill, New York (2001) 9.1-9.32.
[11]. EPA Pesticide Fact Sheet: Cyfluthrin. 1987. Fact Sheet No. 164 EXTOXNET. 2001. Cyfluthrin. Available online http://ace.orst.edu/cgi-bin/mfs/01/pips/cyfluthr.html
[12]. Adams K J, Chavasse DC, Mount DL, Carneiro IA, Curtis CF (2002) Comparative insecticidal power of three pyrethroids on netting. Med Vet Entomol 16:106–108.
[13]. Farm Chemical Handbook. 1993. Meister Publishing Company. Vol. 79.
[14]. Hamman, I. And R. Fuchs. 1981. ®Baythroid, a new insecticide. Pflanzenschutz- Nachrichten, 34(2):121-151.
[15]. Bradbury, S.P., Coats, J.R., 1989. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contam. Toxicol. 108, 133–177.
[16]. Benli, A.C.K., 2005. Investigation of acute toxicity of cyfluthrin on tilapia fry (Oreochromis niloticus L. 1758). Environ. Toxicol. Pharmacol. 20, 279–282.
[17]. Al-Makkawy, H.K., Madbouly, M.D., 1999. Persistence and accumulation of some organic insecticides in Nile water and fish. Resour. Conser. Recycl. 27, 105–115.
[18]. Wolansky, M.J., Harrill, J.A., 2008. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. Neurotoxicol. Teratol. 30, 55–78.
[19]. Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Bulletin of Marine Pollutants, 42: 656-666.
[20]. Kim, J., Chehade, J., Pinnas, J.L., Arshag, D., Mooradian, A.D., 2000. Effect of select antioxidants on malondialdehyde modification of proteins. Nutrition 16, 1079–1081.
[21]. Meister, R.T. 1995. Farm Chemicals Handbook '95. Meister Publishing Company. Willoughby, OH.
[22]. Veeraiah, K. and M.K. Durga Prasad: Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish. Labeo rohita (Hamilton). Proc. Acad. Environ. Biol., 7, 143-148(1998).
[23]. Tilak KS, Satyavardan K, Thathaji PB (2003). Biochemical changes induced by Fenvalerate in the freshwater fish *Channa punctatus*. J. Ecotoxicol. Environ. Monit., 13: 261-270.
[24]. Tripathi, G. and Verma, P. 2004. Fenvalerate induced changes in a catfish, Clarias batrachus: metabolic enzymes, RNA and protein. Comp. Biochem. Physiol., 138C: 75-79.
[25]. Sakr, S.A., S.M. Jamal and AI lail: Fenvalerate induced histopathological and histochemical changes in the Liver of the cat fish Clarias Gariepinus. J. Appl. Sci. Res., 1, 263-267 (2005).
[26]. Babu velmurugan, Mariadoss selvanayagam, Elif I. Cengiz and Erhan Unlu: The Effects of Fenvalerate on different tissues of fresh water fish Cirrhinus mrigala. J. Environ. Sci. Hlth., Part-B, 42, 157-163 (2007).
[27]. Ramaneswari, K. and L.M. Rao: Influence of endosulfan and monocrotophos exposure on the activity of NADPH cytochrome C reductase (NCCR) of Labeo rohita (Ham). J. Environ. Biol., 29, 183-185 (2008).
[28]. Majumdar, T.N. and A. Gupta: Acute toxicity of endosulfan and malathion on Chironomus ramosus (Insecta : Diptera : Chironomidae) from north Cachar hills, Assam, India. J. Environ. Biol., 30, 469-470 (2009).
[29]. Madan, V.K., Singh, R., Kumari, B., Naresh, J.S., Kathpal, T.S. 2000. "Dissipation of lindane and fenvalerate residues in chickpea (Cicer arietinum L.) under Indian climatic conditions". Ecotoxicology and Environmental Safety. 46: 163-166.

© 2019, IJSRBS All Rights Reserved

[30]. Jagan, P., Reddy, M. S. and Rao, A. P. 1989. Effect of certain insecticides on the freshwater fish *Cyprinus carpio carpio*, L. *Indian Journal of Environmental Biology*, 10 (2): 135-138.
[31]. Tandon S.S., Srivastava P.P., Mukherjee S.C. and Saharan N., 2005. Acute toxicity of synthetic pyrethroids to Indian major carp, Catla catla L., Bull Environ Contam Toxicol, 74:60-613.
[32]. Singh, S. P., Chaudhary, R. and Gaur, K. K. 2007. A study of toxicity and behavior of freshwater fish, Channa punctatus (Bloch) after intoxication of carbamate and synthetic pyrethroid fenvalerate. J. Ecophysiol. Occup. Hlth. 7: 41-45.
[33]. Bradbury S.P., Coats J.R. and Mckim J.M., 1985. Differential toxicity and uptake of two fenvalerate formulations in fathead minnows (Pimephales promelas). Environ. Toxicol Chem., 4:533-541.
[34]. Chapman, R.A., Tu, C.M., Harris, C.R and Cole, C. 1981. Persistence of five pyrethroid insecticides on Rainbow trout. *Bull. Environ. Contam. Toxicol. 26 :* 513-519.
[35]. Chapman, R.A. and Harris, C.R. 1981. Persistance of four pyrethroid insecticides in sterile and natural, mineral and organic soil, *Bull. Environ. Contom. Toxicol*, 20: 513-519.
[36]. Miyamoto J (1976): Degradation metabolism and toxicity of synthetic pyrethroid. Environmental Health Perspectives, 14,15-28.
[37]. Casida JE, Benett JP, Vickery BH, Gammon DW(1983): Mechanism of selective action of pyrethroid insecticide. Annual Review of Pharmacology and Toxicology, 23, 413-438.
[38]. Finney DT. Probit Analysis, 3rd edit. Cambridge University Press. London, 1971
[39]. Smith TM, Stratton GW (1986) Effects of synthetic pyrethroid insecticides on nontarget organisms. Res Rev 97:93–119.
[40]. Schneider, W.C. (1957) Determination of Nucleic Acids in Tissues by Pentose Analysis. In: Colowick, S.P. and Kaplan, W.O., Eds., Methods in Enzymology, Academic Press, New York, 680-684.
[41]. shodhganga.inflibnet.ac.in/bitstream/10603/69100/9/09_chapter%202.pdf.
[42]. Walton D G, Acton A B and Stich H F (1984) DNA repair synthesis following exposure to chemical mutagens in primary liver, stomach and intestinal cells isolated from rainbow trout. *Cancer Res*. 44, 1120-1121.
[43]. Espina N G and Wesis P (1995) DNA repair in fish from polluted estuaries. *Mar. Environ. Res.* 39, 309-312.
[44]. Haqqi, T.M. and Adhami, U.M. 1979. Effect of apholate (ENT 26216, NSC 26812) on nucleic acids, total protein content and activity levels of aminotransferases (GOT, GPT), alkaline phosphatase and 5-nucleotidase in liver of male albino rats. Ind. J. Exp. Biol., 17: 488- 492.
[45]. Durairaj, S. and Selvarajan, V.R.1992. Influence of quinalphos, an Organophosphorous Pesticide, on the Biochemical constituents of the tissues of fish, Oreochromis mossambicus J.Environ. Biol. 13(3): p. 181-185.
[46]. Abou-Donia, M.B., Lapadul, D.M. and Carrington, C.D. 1988. Biochemical methods for assessment of neurotoxicity. In: Perspectives in basic and applied toxicology (Ed: B. Ballantyne). Butterworth & Co. Ltd., London, 1-30.
[47]. Singh, K.S., Singh, S.K.S. and Yadav, R.P. 2010.Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost Colisa fasciatus at different seasons. World J. Zool., 5(7): 25-32.
[48]. Van Loon, A.A.W.M., Groenendijk, R.H., Van DerShcanslohman, P.H.M. and Bran, R.A. 1991. Detectionof induced damage in DNA in human blood exposed to ionic radiation at biologically relevant doses. Int. J. Rad. Biol., 59: 651-660.
[49]. Gowri, B., Govindassamy, P. and Ramalingam, V. 2013. Influence of Cypermethrin on DNA and RNA Content in Different Organs of Freshwater Fish *Cyprinus carpio*. Iran. J. Pharm. Sci., 9(3): 1-10.
[50]. Veeraiah, K., Vivek, Ch., Srinivas Rao, P. and Venkatrao, G. 2013. Biochemical Changes induced by Cypermethrin (10% EC), A Pyrethroid Compound in Sublethal and Lethal Concentrations to the Freshwater Fish *Cirrhinus mrigala* (Hamilton). J. Atom. Mol., 3(6): 625-634.
[51]. Thakur, A.C. and Kakde, V.R. 2012. Influence of Cypermethrin on DNA, RNA and RNA/DNA Ratio in Muscles of the Freshwater Fish *Channa punctatus*. Int. Interdisci. Res. Jour., 2: 38-42.
[52]. Tiwari, S., Tiwari, R. and Singh, A. 2012. Impact of Cypermethrin on Fingerlings of Common Edible Carp (*Labeo rohita*). The Scient. World Jour., 1: 1-7.
[53]. Singh, K.S., Singh, S.K.S. and Yadav, R.P. 2010.Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroid) against freshwater teleost Colisa fasciatus at different seasons. World J. Zool., 5(7): 25-32.
[54]. Vasantharaja, C., Pugazhendy, C., Meenaambai, M., Prabukaran, S. and Dar, N.A. 2013. Properties of *Cardiospermum halicacabum* against the toxic effect of cypermethrin in the quantification of nucleic acids in the freshwater fish *Cirrhinus mrigala* (Hamilton). Int. Res.J. Pharm., 4(1): 280-283.
[55]. Raksheskar, G.A. 2012. Influence of cypermethrin on DNA, RNA and RNA/DNA ratio in gills of the freshwater fish Channa striatus. Biosci. Disc., 3: 17-19.
[56]. Kumar, A., Sharma, B. and Pandey, R.S. 2007. Preliminary evaluation of the acute toxicity of cypermethrin and λ- cyhalothrin to *Channa punctatus*. Bull. Environ. Contam. Toxicol., 79: 613- 616.
[57]. Tripathi, G, ., (2002): J. Environ Bio.vol-23, issue 2, pg 143–146.
[58]. Maruthanayagam, C, and Sharmila, G, (2004),Nature Env. Polln. Techn. Vol-3, issue 4, pg 491–494.
[59]. Tripathi, P. K,., (2003), Asian fisheries Sci. issue 16, pg 349–359.

AUTHORS PROFILE

Dr S.Mohammed Ghouse M.Sc.,SLET., Ph.D, ., Zoology from Sri Krishna devaraya University, anantapurum, Andhra Pradesh, India in 1997,2000, 2006. He is currently working as a Reader in Department of Zoology , Osmania College, Kurnool since 1997. Life Member of The Indian Science Congress Association, Kolkata, India, Life Member of Indian Society of Cell Biology, India 2010, Life Member of ISCAP Tirupati, India –December 2010, Member of ISZS , CHINA 2010, Received State Teacher Award on 5th September 2015 A.P Government fromhon.CM Chandrababu Naidu He has published more than 45 research papers in reputed National and International journals including Thomson Reuters (SCI & Web of Science) and conferences also, available online and authored 3 books . His main research work focuses on Environmental biology, toxicology, avian biology etc. He has 22 years of teaching experience and 15 years of research experience.

© 2019, IJSRBS All Rights Reserved

International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 4, Issue 4, pp: 155-161, 2019
https://doi.org/10.5281/zenodo.3356818
http://www.ijzab.com

Research Article

IMPACT OF SYNTHETIC PYRETHROIDS CYFLUTHRIN AND FENVALERATE ON ACETYLCHOLINESTERASE ACTIVITY(ACHE) OF FRESH WATER FISH *GAMBUSIA AFFINIS*

S. Mohammed Ghouse*

Department of Zoology, Osmania College, Kurnool- 51800, Andhra Pradesh, India.

Article History: Received 24th June 2019; Accepted 28th July 2019; Published 30th July 2019

ABSTRACT

Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. These pesticides through the process of biomagnification increase their concentration at the top trophic level of the food chain, including the in humans. *Gambusia affinis* was subjected to sub lethal concentrations of cyfluthrin and fenvalerate from 24 to 96 hours Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

Keywords: Pesticides, Acetylcholinesterase enzyme, Decrease, tissues, *Gambusia affinis*.

INTRODUCTION

Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. Under the U.S. Federal Environmental Pesticide Control Act, the term Pesticide has been defined to include any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (Barse *et al*., 2006). These pesticides by different routes finally reach the aquatic systems affecting the aquatic organisms (Santhakumar *et al*., 1999). Pesticides are hazardous to the biodiversity including the man. Pesticides have detrimental effects on behaviour, reproduction, growth, hormonal regulation etc of the organisms .These pesticides through the process of biomagnifications increase their concentration at the top trophic level of the food chain, including the in humans. Fishes are usually exposed to highly contaminated water, which are in areas where waste water dilution rate is low (Bernet *et al*., 1999). Fish play a vital role in the toxicity testing for ecological effects as the most employed organisms for bio monitoring. In fish, the principle mode of uptake of pesticides is through the gills (Johnson, 1968), body surface and gastro-intestinal system (Costa & Murphy, 1983), through integument (Bowes, 1972) and by the ingestion of contaminated food (Macek *et al*., 1969). Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

*Corresponding Author: Dr. S. Mohammed Ghouse, Reader, Department of Zoology, Osmania College, Kurnool- 51800, Andhra Pradesh, India. Email: syed0002001@gmail.com, Mobile: +91 9501603265

MATERIALS AND METHODS

Fish collection and laboratory conditions

Adult fresh water teleost fishes *Gambusia affinis* with weight (0.7-1.5 gm), length (2.3 - 3.2 cm) from both sexes were selected randomly for the experimental studies were collected from water bodies and fisheries department of Kurnool district, Andhra Pradesh, India. The collected fish were transported in polythene bags filled with half water with least disturbance. They are kept in aquarium tanks containing well aerated de-chlorinated water to acclimatize to the laboratory conditions, with physic - chemical characteristics: temperature 24 ± 2°C, pH 7.1 ± 0.2 at 24°C, dissolved oxygen 9.6 ± 0.8 mg/L, carbon dioxide 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg as $CaCO_3$/L, phosphate 0.39 ± 0.002 µg/L, salinity in different cement tanks for one week. To prevent fungal infection the fish tanks were washed with potassium permanganate solution. Dead fish were removed as soon as possible to avoid water fouling. Fishes were fed daily on commercial fish food. The unused food is removed daily and water was renewed daily .Test fishes were screened critically for any signs of stress, disease, physical damage and mortality. Injured, severely, diseased fishes were discarded. The fishes were exposed to sublethal concentrations for treated and control period of 96 hours. A control group was maintained with identical environment. The toxicant water as well as normal water is renewed every day. The fish were sacrificed from both experimental and control groups on 96 hours.

Selection of pesticides

In the present investigation two synthetic pyrethroids were selected namely Cyfluthrin and Fenvalerate.

Characteristics of Cyfluthrin

Cyfluthrin, cyano (4-fluoro-3- phenoxyphenyl) methyl-3-(2,2dichloroethenyl)-2,2-imethylcyclopropanecarboxylate) is a broad spectrum synthetic type II pyrethroid insecticide, Cyfluthrin stable at room temperature, but sensitive to sunlight, CAS Registry Number: 68359-37-5, Chemical Class: Synthetic pyrethroid, Use: Broad spectrum insecticide for a variety of agricultural crops, Toxicity rating: Moderately toxic, Signal Words: Caution. Warning - Danger, Health Effects: it acts as a neurotoxic poison, causes organ inflammation and induces skin paresthesia as per the animal studies, Environmental Effects: Cyfluthrin is extremely toxic to bees, fish and other aquatic organisms. (Source: Cyfluthrin chemical watch Factsheet, August 2007).

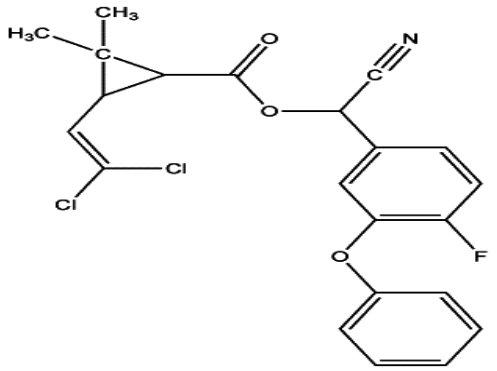


Figure 1

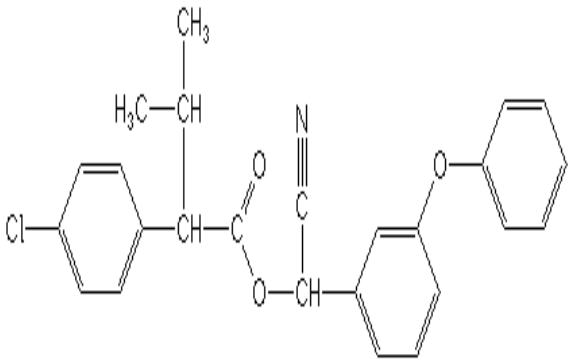


Figure 2

Figure 1. Cyfluthrin cyano (4-fluoro-3-phenoxyphenyl) methyl-3-(2,2-dichloroethenyl)-2, 2-dimethyl-cyclopropanecarboxylate. **Figure 2**. IUPAC name: (RS)-a-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate Chemical Abstracts name: cyano (3-phenoxyphenyl) methyl 4-chloro (1-methylethyl) benzene acetate.

Fenvalerate

Fenvalerate, is a clear viscious yellow liquid with a mild odor, a synthetic pyrethroid. Fenvalerate a synthetic pyrethroid is an effective against the bollworms, aphids, weevils, leaf hoppers, stem pod, fruit borers etc which are common pests of agriculture. The technical grade containing 92% fenvalerate and 8% related compounds were obtained from Searle India ltd, Mumbai. Generic name (IUPAC name): (RS)-alpha-Cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate, Common name: Fenvalerate, Trade names: Pydrin, Sumicidin, Belmark, CAS number: 51630-58-1, EPA Code: 109301, Year of initial registration: 1978.

Experimental design

Group 1: Fish exposed to tap water observed for 24 to 96 Hours (Untreated control). Group 2: Fish exposed to sublethal concentration of cyfluthrin of 24 to 96 hours. Group 3**:** Fish exposed to sublethal concentration of fenvalerate of 24 to 96 hours. The toxicity studies will conducted by using the technical grade formulation available formulation of Fenvalerate and Cyfluthrin. Solutions of desired concentrations were prepared in 95% acetone to get the stock solution as well as working solution as 100mg/100ml and 1mg/1ml of toxicant chemical Fenvalarate and Cyfluthrin in both technical formulation use of acetone in control as recommended by

EPA will be followed**.** The acclimatized fishes were kept in cement tanks and ten fishes in two groups control fishes will be kept in dechlorinated water only. Each set of experiment was replicated six times. Mortality was recorded every 24 h during the observation period of 96h.

Statistical analysis

The data obtained from the quantitative study were expressed as the mean ± S.E. The mean values were calculated from 6 individual observations; P-values were calculated by the students't' test. The two mean values obtained were considered significant from each other with standard error.

Estimation of Acetylcholine esterase activity (AChE) activity

The method of AChE activity estimation is popularly known as Ellman's method (Ellman *et al*., 1961) named after George Ellman who developed this method in 1961 The esterase activity is measured by providing an artificial substrate, acetylthiocholinelodide (ACI). Thiocholine released because of the cleavage of ACI by AChE is allowed to react with the -SH reagent 5, 5'- dithiobis- (2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm. Assay was performed by adding 2.9ml of O.lmM sodium phosphate buffer (p.H-8) to 50ml of supernatant of different region of brain and then incubated at 37 for 5 minutes. After incubation 40ul of Acetylthiocholine iodide (154.38mM), 10ul of DTNB (10mM) was added, mixed by inverting cuvette and absorbance was taken at 412nm for 150 sec at interval of 30sec using UV spectrophotometer. The extinction coefficient of the thionitrobenzoic acid is 1.36 x 104/ molar/ centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is than taken as direct estimate of AChE activity.

RESULTS AND DISCUSSION

In the present observation when Gambusia *affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed significant decrease in the levels of AChE activity content in the gill when compared with the control group. The percent changes over the control with cyfluthrin (group-2) are - 10.21, - 18.97, - 28.62, - 36.08 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decrease in AChE activity over the control are - 12.41, - 20.67, - 31.43, - 40.96 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3). In the present study the observed values of AChE activity in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a significant decline ,when compare with the control group. The percent decline with cyfluthrin (group-2) are -8.89, - 16.23, - 26.72, - 34.67 for 24, 48, 72 and 96 hours respectively. The percent decrease with fenvalerate (group-3) are - 9.71, - 17.67, - 29.60, - 34.11 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

Pesticides deliberately added to the environment for eradication of pests but they are entering in to the environment affecting the non target organism like fishes. So fishes are used widely for the bioassay of these pesticides .Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

This enzyme is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system (Silman & Sussman, 2005; Soreq & Seidman, 2001). AChE catalyses the hydrolysis of Ach and plays a role in cholinergic transmission by reducing the concentration of acetylcholine (ACh) in the junction to regulate nervous transmission (Kopecka *et al*., 2004). AChE inhibition of results in an accumulation of acetylcholine, which leads to tetany, paralysis, and eventual death due to the continuous and excessive stimulation of the nerve/muscle fibers. AChE inhibition of results to a variety of neurotoxic effects and decreased cholinergic transmission due to the buildup of acetylcholine within the nerve synapses (Mileson *et a*l., 1998). AChE inhibition results in functionally vital organs like gill, muscle and liver lead to impaired critical neurophysiological activity and block sodium channels of nerve filaments, thereby lengthening the depolarization phase. Pyrethroids delay the closing of the gate that allows sodium flow (Vijverberg & vanden Bercken, 1990) and thus, multiple nerve impulses rather than the usual single one occur. In turn, these impulses release the neurotransmitter ACh, which stimulates other nerves (Eells *et al*., 1992) ultimately resulting in buildup of ACh within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Mushigeri & David, 2005). It is also known pyrethroid compounds which inhibit AChE activity were known to disrupt the normal behavioral patterns in the effected animals (De la Torre *et al*., 2002). In *Cyprinus carpio* and *Cnesterodon decemm-aculatus* showed reduce level of acetylcholinesterase (Roos *et al*., 1980).

The AChE activity in the tissues of kidney in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 12.65, - 21.37, - 28.99, - 43.33 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 13.88, - 23.85, - 31.44, - 37 for 24, 48, 72 and 96 hours

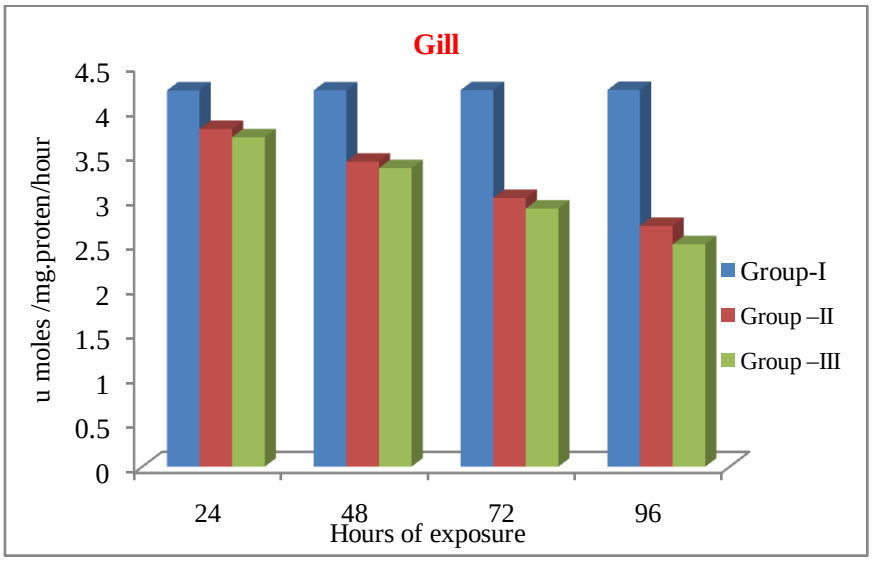
respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

The levels of AChE activity in the muscle shown a declining trend in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of decline in fish exposed to cyfluthrin (group-2) are - 8.46, - 15.86, - 24.59, - 33.11 for 24, 48, 72 and 96 hours respectively. The decline percentage in fish exposed to fenvalerate (group-3) are - 9.60, - 16.56, - 23.41, - 34.62 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

Table 1: Variations of Acetylcholine esterase (AChE) (umole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	Hours of exposure 24	48	72	96
Gill	Group-I Control	4.220 ± 0.056	4.222 ± 0.057	4.224 ± 0.058	4.226 ± 0.059
	Group –II- Cyfluthrin	3.789** ± 0.034	3.421** ± 0.033	3.015** ± 0.036	2.701** ± 0.034
	% COC	% - 10.21	% - 18.97	% - 28.62	% - 36.08
	Group –III- Fenvalerate	3.696** ± 0.044	3.349** ± 0.046	2.896** ± 0.046	2.495** ± 0.047
	% COC	% - 12.41	% - 20.67	% - 31.43	% - 40.96
Liver	Group-I Control	3.540 ± 0.071	3.542 ± 0.073	3.543 ± 0.076	3.544 ± 0.079
	Group –II- Cyfluthrin	3.225** ± 0.048	2.967** ± 0.051	2.596** ± 0.053	2.315** ± 0.061
	% COC	% -8.89	% - 16.23	% - 26.72	% - 34.67
	Group –III- Fenvalerate	3.196** ± 0.027	2.916** ± 0.028	2.494** ± 0.031	2.335** ± 0.034
	% COC	% - 9.71	% - 17.67	% - 29.60	% - 34.11
Kidney	Group-I Control	3.960 ± 0.029	3.962 ± 0.029	3.963 ± 0.034	3.962 ± 0.037
	Group –II- Cyfluthrin	3.459** ± 0.066	3.115** ± 0.068	2.814** ± 0.072	2.245** ± 0.076
	% COC	% - 12.65	% - 21.37	% - 28.99	% - 43.33
	Group –III- Fenvalerate	3.410** ± 0.091	3.017** ± 0.092	2.717** ± 0.092	2.496** ± 0.096
	% COC	% - 13.88	% - 23.85	% - 31.44	% - 37
Brain	Group-I Control	4.581 ± 0.081	4.583 ± 0.082	4.583 ± 0.085	4.584 ± 0.086
	Group –II- Cyfluthrin	4.193** ± 0.062	3.856** ± 0.065	3.456** ± 0.072	3.066** ± 0.076
	% COC	% - 8.46	% - 15.86	% - 24.59	% - 33.11
	Group –III- Fenvalerate	4.141** ± 0.074	3.824** ± 0.077	3.510** ± 0.079	2.997** ± 0.080
	% COC	% - 9.60	% - 16.56	% - 23.41	% - 34.62

Values are mean ± S.E Mean of six individual observations and student t-test. Significant at * P<0.05; Significant at * P<0.01 levels (+, -) denotes decreased and increased. %COC (change over control).



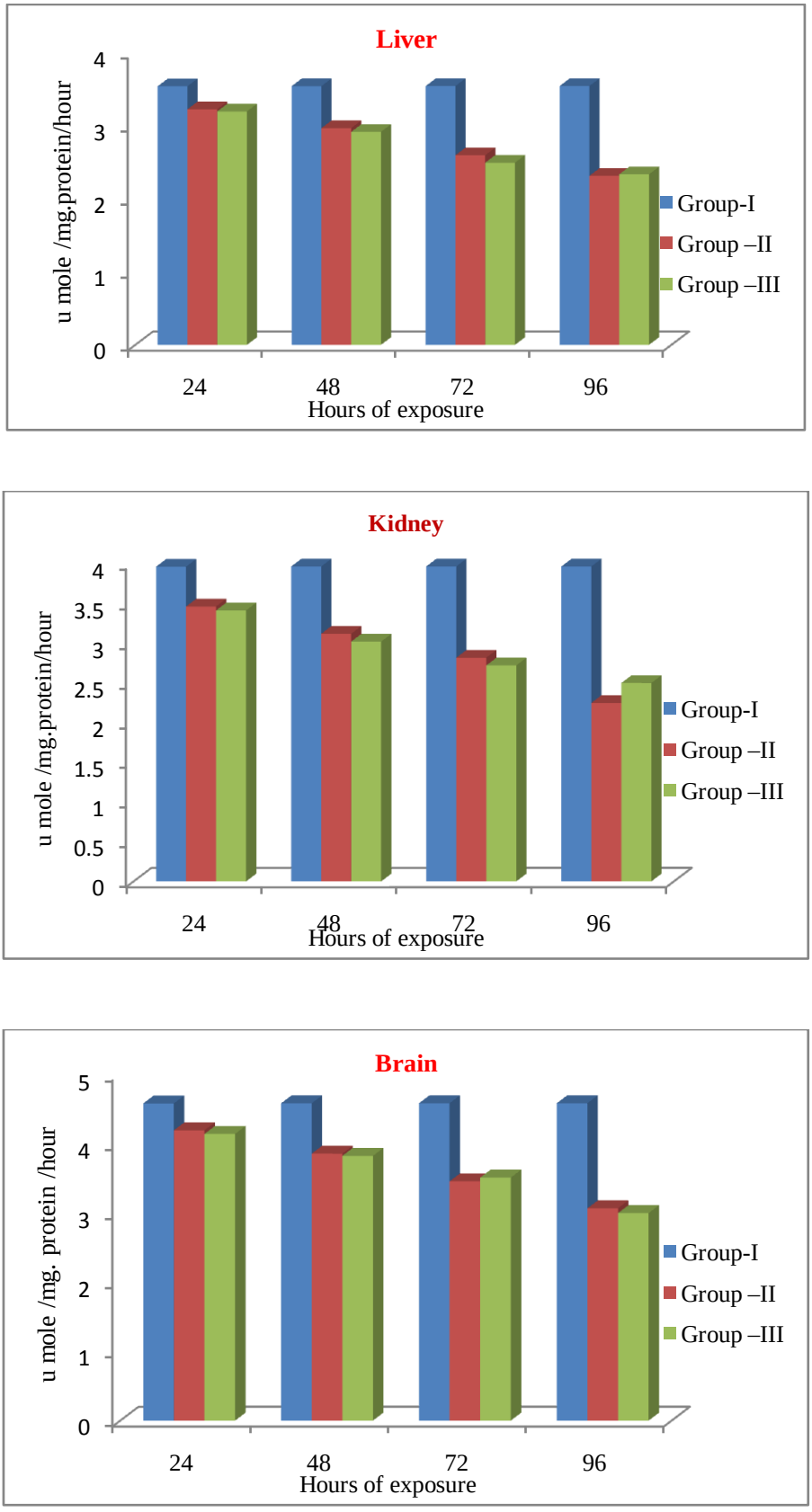


Figure 3. Changes in the level of Acetylcholine esterase (AChE) (u mole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours.

It has been established that the AChE enzyme unit consists of a negative subsite, which attracts the quaternary group of choline through both columbic and hydrophobic forces and an esterase subsite, where nucleophilic attack occurs on the acetyl carbon of the substrate (Key *et al*., 1998). OPs inhibitory action resulted in decrease in AChE level was probably due to the impairment of neural transmission. The decrease in AChE activity when grass shrimp exposed to sub lethal concentration of malathion, (Modesto & Martinez, 2010) decreased AChE activity in *Prochilodus lineatus* in the brain and the muscle of when exposed to glyphosate (Reddy *et al*., 1992).

Similar reports which collaborates our findings in the inhibition of AChE activity due to pyrethroids exposure is reported in *Cyprinus carpio* (Somnuek *et al*., 2007; Szegletes *et al*., 1995).Similar observation are made in the alterations of levels of the AChE activity in the brain, liver, muscle and gill tissues of hybrid catfish, *Clarias macrocephalus* and *Clarias gariepinus* exposed to a sublethal concentration of chlorpyrifos and a carbamate, carbaryl for 4 days and reported that AChE inhibition increased rapidly with insecticide concentration (Rodrıguez fuentes & Gold bouchot, 2000). AChE inhibition is observed under cypermethrin exposure in different fishes (Ahmad *et al*., 2000; Bretaud *et al*., 2000; Cattaneo *et al*., 2008; Ferrari *et al*., 2007; Lionetto *et al*., 2003; Van Cong *et al*., 2009; Wang *et al*., 2009).

CONCLUSION

It can be concluded that there is correlation between fish cyfluthrin and fenvalerate exposure and decline in the Acetylcholinesterase enzyme contents in selected tissues. Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Osmania College, Kurnool-51800, Andhra Pradesh, India for the facilities provided to carry out this research work.

REFERENCES

Ahmad, S., Scopes, R. K., Rees, G. N., & Patel, B. (2000). Saccharococcus caldoxylosilyticus sp. nov., an obligately thermophilic, xylose-utilizing, endospore-forming bacterium. *International Journal of Systematic and Evolutionary Microbiology, 50*(2), 517-523.

Barse, A., Chakrabarti, T., Ghosh, T., Pal, A., & Jadhao, S. (2006). One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pesticide Biochemistry and Physiology, 86*(3), 172-179.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases, 22*(1), 25-34.

Bowes, G. W. (1972). Uptake and Metabolism of 2, 2-bis-(p-Chlorophenyl-1, 1, 1-trichloroethane (DDT) by Marine Phytoplankton and Its Effects on Growth and Chloroplast Electron Transport. *Plant Physiology, 49*(2), 172-176.

Bretaud, S., Toutant, J.P., & Saglio, P. (2000). Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*). *Ecotoxicology and Environmental Safety, 47*(2), 117-124.

Cattaneo, R., Loro, V., Spanevello, R., Silveira, F., Luz, L., Miron, D., Clasen, B. (2008). Metabolic and histological parameters of silver catfish (*Rhamdia quelen*) exposed to commercial formulation of 2, 4-dichlorophenoxiacetic acid (2, 4-D) herbicide. *Pesticide Biochemistry and Physiology, 92*(3), 133-137.

Costa, L., & Murphy, S. (1983). [3H] Nicotine binding in rat brain: alteration after chronic acetylcholinesterase inhibition. *Journal of Pharmacology and Experimental Therapeutics, 226*(2), 392-397.

De la Torre, F., Ferrari, L., & Salibian, A. (2002). Freshwater pollution biomarker: response of brain acetylcholinesterase activity in two fish species. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 131*(3), 271-280.

Eells, J. T., Bandettini, P. A., Holman, P. A., & Propp, J. M. (1992). Pyrethroid insecticide-induced alterations in mammalian synaptic membrane potential. *Journal of Pharmacology and Experimental Therapeutics, 262*(3), 1173-1181.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88-95.

Ferrari, A., Venturino, A., & de D'Angelo, A. M. P. (2007). Effects of carbaryl and azinphos methyl on juvenile rainbow trout (Oncorhynchus mykiss) detoxifying enzymes. *Pesticide Biochemistry and Physiology, 88*(2), 134-142.

Johnson, D. W. (1968). Pesticides and fishes-a review of selected literature. *Transactions of the American Fisheries Society, 97*(4), 398-424.

Key, P. B., Fulton, M. H., Scott, G. I., Layman, S. L., & Wirth, E. F. (1998). Lethal and sublethal effects of malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. *Aquatic Toxicology, 40*(4), 311-322.

Kopecka, J., Rybakowas, A., Barˇsiene, J., & Pempkowiak, J. (2004). AChE levels in mussels and fish collected off Lithuania and Poland (southern Baltic). *Oceanologia, 46*(3).

Lionetto, M., Caricato, R., Giordano, M., Pascariello, M., Marinosci, L., & Schettino, T. (2003). Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. *Marine Pollution Bulletin, 46*(3), 324-330.

Macek, K. J., Hutchinson, C., & Cope, O. B. (1969). The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. *Bulletin of Environmental Contamination and Toxicology, 4*(3), 174-183.

Mileson, B. E., Chambers, J. E., Chen, W., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., Karczmar, A. G. (1998). Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicological Sciences, 41*(1), 8-20.

Modesto, K. A., & Martinez, C. B. (2010). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere, 81*(6), 781-787.

Mushigeri, S., & David, M. (2005). Fenvalerate induced changes in the Ach and associated AchE activity in different tissues of fish *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. *Environmental Toxicology and Pharmacology, 20*(1), 65-72.

Reddy, P. M., Philip, G. H., & Bashamohideen, M. (1992). Regulation of AChE system of freshwater fish, *Cyprinus carpio*, under fenvalerate toxicity. *Bulletin of Environmental Contamination and Toxicology, 48*(1), 18-22.

Rodrıguez-Fuentes, G., & Gold-Bouchot, G. (2000). Environmental monitoring using acetylcholinesterase inhibition in vitro. A case study in two Mexican lagoons. *Marine Environmental Research, 50*(1-5), 357-360.

Roos, B. O., Taylor, P. R., & Si, P. E. (1980). A complete active space SCF method (CASSCF) using a density matrix formulated super-CI approach. *Chemical Physics, 48*(2), 157-173.

Santhakumar, M., Balaji, M., & Ramudu, K. (1999). Effect of sublethal concentrations of monocrotophos on erythropoietic activity and certain hematological parameters of fish Anabas testudineus (Bloch). *Bulletin of Environmental Contamination and Toxicology, 63*(3), 379-384.

Silman, I., & Sussman, J. L. (2005). Acetylcholinesterase:'classical'and 'non-classical'functions and pharmacology. *Current Opinion in Pharmacology, 5*(3), 293-302.

Somnuek, C., Cheevaporn, V., Saengkul, C., & Beamish, F. W. H. (2007). Variability in acetylcholinesterase upon exposure to chlorpyrifos and carbaryl in hybrid catfish. *Science Asia, 33*, 301-305.

Soreq, H., & Seidman, S. (2001). Acetylcholinesterase-new roles for an old actor. *Nature Reviews Neuroscience, 2*(4), 294.

Szegletes, T., Balint, T., Szegletes, Z., & Nemcsok, J. (1995). In vivo effects of deltamethrin exposure on activity and distribution of molecular forms of carp AChE. *Ecotoxicology and Environmental Safety, 31*(3), 258-263.

Van Cong, N., Phuong, N. T., & Bayley, M. (2009). Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). *Ecotoxicology and Environmental Safety, 72*(3), 699-703.

Vijverberg, H. P., & vanden Bercken, J. (1990). Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Critical Reviews in Toxicology, 21*(2), 105-126.

Wang, C., Lu, G., Cui, J., & Wang, P. (2009). Sublethal effects of pesticide mixtures on selected biomarkers of Carassius auratus. *Environmental Toxicology and Pharmacology, 28*(3), 414-419.